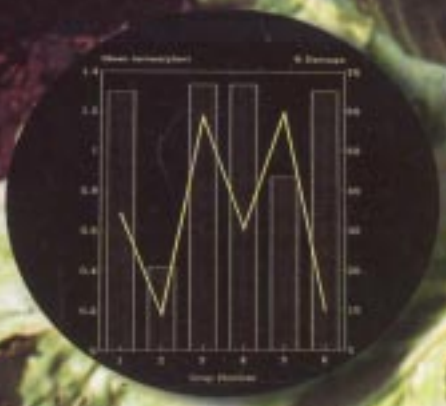


THE MANAGEMENT OF DIAMONDBACK MOTH AND OTHER CRUCIFER PESTS

Proceedings of the Third International Workshop,
Kuala Lumpur, Malaysia
29 October – 1 November 1996

Malaysian Agricultural Research and Development Institute (MARDI)

Malaysian Plant Protection Society (MAPPS)

THE MANAGEMENT OF DIAMONDBACK MOTH AND OTHER CRUCIFER PESTS

Proceedings of the Third International Workshop,
Kuala Lumpur, Malaysia
29 October – 1 November, 1996

Editors
A. Sivapragasam
W. H. Loke
A. K. Hussan
G. S. Lim

Malaysian Agricultural Research and Development Institute (MARDI)
P. O. Box 12301, G.P.O., 50774 Kuala Lumpur

Malaysian Plant Protection Society
P. O. Box 12351, G.P.O., 50774 Kuala Lumpur

First Published 1997

Perpustakaan Negara Malaysia Cataloguing-in-Publication Data

International Workshop on the Management of Diamondback Moth and Other Crucifer Pests (3rd : 1996 : Kuala Lumpur)
The management of diamondback moth and other crucifer pests : proceedings of the Third International Workshop, Kuala Lumpur, Malaysia, 29 October - 1 November, 1996 / editors A. Sivapragasam ... [et al.].
Includes index
ISBN 967-936-335-X
1. Yponomeutidae--Integrated control--Congresses.
2. Diamondback moth--Integrated control--Congresses.
3. Cruciferae--Diseases and pests--Congresses.
I. Sivapragasam A. II. Institut Penyelidikan dan Kemajuan Pertanian Malaysia.
III. Title.
632.78

Published and printed by:
Malaysian Agricultural Research and Development Institute (MARDI)
Ministry of Agriculture, Malaysia

CONTENTS

DECISION TOOLS FOR DBM

CHEMICAL CONTROL

PESTICIDE RESISTANCE MECHANISMS AND RESISTANCE MANAGEMENT STRATEGIES

DEVELOPMENT AND IMPLEMENTATION OF IPM

POSTER PAPERS

FOREWORD

Cruciferous vegetables are economically important crops for the Asian populace as they form an essential part of the diet. On a worldwide basis, the extent of cultivation of these vegetables is about 2.2 million ha with fifty percent of the production coming from Asia. However, despite the advancement in pest control technologies, pests such as the ubiquitous diamondback moth (DBM), still pose a major constraint for cruciferous vegetable production in many countries. In recent years, DBM has become the most destructive insect of cruciferous plants throughout the world with annual costs for managing it estimated to be in the region of US$1 billion. This pest now occurs wherever crucifers are grown and is believed to be the most universally distributed of all Lepidoptera. Despite the numerous attempts made to control this pest, outbreaks and poor control, *etc.* are still being reported in many countries. Of pertinence here is the increasing resistance development of this pest against intensively-used pesticides and the microbial agent, *Bacillus thuringiensis*. Associated with indiscriminate applications of pesticides is the greater concern of governments towards the health of users and the safety of the produce and the environment.

Crucifer pests, in particular the diamondback moth, have been the key subjects of two widely attended international workshops held in Taiwan. The organization of the third workshop was, therefore, a timely one and an anticipated development. This third workshop provided a useful platform to deliberate the recent advances made in the management of the DBM and other associated pests of crucifers. Of particular significance was the sharing of information between more than 160 participants from over 20 countries and the underscoring of approaches that minimize the use of `hard' pesticides and increase the use of biologically-based technologies. This proceedings, which will complement the two earlier ones, thus offers a unique collection of experiences on worldwide endeavours to manage the DBM and other crucifer insect pest problems.

MARDI is pleased to have joined hands with the Malaysian Plant Protection Society (MAPPS), Centre for Agriculture and Biosciences International (CABI)–Asia Regional Office, Department of Agriculture, Malaysia and the Silwood Centre for Pest Management, United Kingdom in successfully organising this workshop. I take this opportunity to thank the Honorable Minister of Agriculture, Malaysia once again for his gracious presence in officiating the opening of the workshop. I also express my gratitude to the various sponsors for their financial support and to all speakers, poster presenters and participants for their participation. Last but not least, my special thanks go to the Editorial Committee and all others involved in the publication of this proceedings for their enduring and tireless efforts.

Dr. Saharan Hj. Anang
Deputy Director General MARDI/
Chairman, Organising Committee
Third International Workshop on the Management
of the Diamondback Moth and Other Crucifer Pests

PREFACE

This proceedings consist of papers presented at the Third International Workshop on the Management of Diamondback Moth and the Other Crucifer Pests which was held in Kuala Lumpur, Malaysia from the 29th October to 1 November 1996. It also includes a special summary highlighting the important points discussed during the workshop.

The workshop papers have been compiled and presented in a standard journal format. As with many other proceedings of this nature, most of the papers received minimal amount of reviewing and editing which have invariably led to variations in standard and style of presentation. However, it is hoped that major factual and typographical errors have been minimized. For some papers involving a major effort of editing, every attempt has been made to retain the original meaning and views of the authors. All claims of commercial products and processes as well as views expressed do not imply endorsement by the editors or the organisers.

Based on the papers presented during the workshop, the proceedings is divided into thematic sections that include lead papers, oral presentations and poster presentations. The oral presentations have been for convenience divided into six major subsections, *viz.*, status of the diamondback moth and other pests of crucifers and their biocontrol, biologically-based technologies, decision tools, chemical control, pesticide resistance mechanisms and resistance management strategies and finally, experiences on the development and implementation of integrated pest management programmes in various countries. In addition, keywords and subject index have been included to facilitate easy reference by the user.

We take this opportunity to thank the Director General of MARDI, the President of the Malaysian Plant Protection Society and the Organising Committee of the workshop headed by Dr. Saharan Hj. Anang for their relentless support and encouragement. A special word of thanks is in order for all authors of papers for their cooperation and the MARDI Publication Unit, *viz* Hjh. Rohani Mahmood, Marina Fatimah Baptist, Siti Fatimah Karim, Azidah Mohd. Yusof, Hamidah Hassan and Zulkhairy Aminuddin for their efforts and cooperation in the formatting and printing of this proceedings. Last, but not least, we acknowledge and accept responsibility for any errors that have not been corrected due to our human fallibility.

Editors

Crucifer insect pest problems: trends, issues and management strategies

G.S. Lim[1], A. Sivapragasam[2] and W.H. Loke[2]

[1]*IIBC Station Malaysia, P.O. Box 210, 43409 UPM Serdang*

[2]*Strategic, Environment and Natural Resources Center, MARDI, P.O. Box 12301, 50774, Kuala Lumpur*

Abstract

Crucifer vegetables are important cultivated crops and are widely grown in many parts of the world, including the highlands in most tropical countries. They are frequently attacked by a number of important insect pests. Some have been a problem for a long time while others have become important only recently. For many, trends are apparent pertaining to their changing status, including other aspects and strategies associated with efforts to counter them. In some cases, they are also closely associated with other agricultural developments and agronomic practices which are driven by commercial interests in vegetable production.

Among the major trends recognised are: (1) A number of pests persisting to be important (e.g., *Plutella xylostella* or diamondback moth (DBM), *Pieris rapae*, *Hellula undalis*), (2) Negative impacts of pesticides continuing to be of major concern where DBM remains a serious problem, (3) Some pests becoming more important, either independently of DBM suppression (e.g., leafminer and *Spodoptera exigua*) or as a consequence of effective DBM control (e.g., aphid and *Crocidolomia binotalis*), (4) DBM becoming increasingly resistant to *Bacillus thuringiensis*, and (5) New cultivation practices emerging, such as rain shelter and hydroponic systems. All these have generated new concerns, additional issues and challenges, and have demanded a review of crucifer cultivation and the associated pest problems, including their management practices and approach. Some major ones include issues of weak extension support, lack of holistic approach in research, incongruence of scientific research with farmers' needs, limited efforts in biological control, increased interest in integrated pest management programmes, organic agriculture, and others.

This paper examines the changing trends and possible reasons, including the management approaches being developed to deal with current chemical control practices. Related issues are appraised along with their implications and follow-up strategies proposed to deal with them are discussed.

Key words: Crucifer pests, trends, issues, management strategies

Introduction

Many cultivated crucifers are important vegetables. They are widely grown in many parts of the world, including the highlands of the tropics. A number of important insect pests frequently attack them (Lim and Di, 1990; Talekar, 1992). Some have been problems for a long time while a few are newly emerging pests. For many of these pests, trends are apparent in relation to their changing status, including other aspects associated with efforts to counter them. In some cases, they are also closely associated with changing agricultural developments driven purely by intensified commercial interests. On the other hand, counter pressures from environmental concerns have played a key role to cushion the impacts from such unilateral commercial interests.

This paper examines the changing trends in the status of crucifer pests and their management, and underlines the possible reasons with particular focus on situations in the developing tropics in Asia. The trends involve mainly those of persistent and emerging insect pests, including trends of pest management approaches being developed to deal with the pests and the escalating problems associated with current chemical control practices. Related issues are appraised along with their implications and the follow-up strategies proposed to deal with them are discussed.

Trends in pest situations

Persistence of major pests

Among the major trends recognised is the persistence of several key pests on crucifers. An excellent example is *Plutella xylostella* or the diamondback moth (DBM) which occurs widely in both the hot and cool areas. Another important pest is the cabbage white butterfly, *Pieris rapae*, which is confined to the cooler locations. However, in the hot tropical lowlands, the webworm *Hellula undalis* has persisted as an important pest in many places. Several other species have also been reported to be important, *viz*, cutworm (*Agrotis ypsilon*), armyworm (*Spodoptera litura*), cabbage head worm (*Crocidolomia binotalis*), flea beetle (*Phyllotreta* spp), and aphids (*Myzus persicae, Brevicoryne brassicae*) *(Table 1)*. However, these do not constantly inflict serious damage, although their presence is often noticeable.

The reasons may differ for different species as to why some of these pests are important. For some, an

important reason is because they are exotic pests without their full complements of effective natural enemies. Specific examples include DBM and the cabbage white butterfly. Even in situations where the natural enemies have been successfully introduced, there exist many cases where the parasites are unable to exert their full impacts due to excessive use of harmful insecticides. Examples are situations of the Cordillera Highlands in the Philippines from 1989–93 and that of the Cameron Highlands in Malaysia in recent years. In general, most of the pests have persisted because of poor understanding of the pest ecology in the tropics, including a lack in ecological approach to managing them. Control efforts have all along been devoted to using and finding more and more effective chemical pesticides. This has led to *pesticide treadmills* occurring in most vegetable areas and the pest problem persisting to be critical. Some newly emergent pests (e.g., leafminers) in several countries (e.g., Indonesia, Malaysia, Philippines, Vietnam) appear to have also fallen into the same condition (*Table 1,* see also subsection on other recent pests).

Emergence of other pests as a consequence of DBM control

Some pests have become more important, apparently as a consequence of effective DBM control. Prominent among these are aphids and the cabbage head worm, *C. binotalis*. Farmers in the Cordillera Highlands (Philippines) have reported that these species are posing a bigger problem after DBM has been successfully suppressed by the parasite, *Diadegma semiclausum*, in conjunction with Farmer Field Schools (FFS). This phenomenon is also recently noted elsewhere (e.g., Indonesia, Malaysia) where some success of DBM control have been achieved.

The reasons for both aphids and the cabbage head worm becoming more important is presently unclear. It could truly be a case of successions by both the species moving into the niche previously occupied by DBM, now that competition of the latter is eliminated. Alternatively, it could be a shift in focus by the farmers to the next important pest problem now that DBM has become less or is no more serious. Or probably, it is a combination of both. Whatever may be the actual reason, the reality is that both these species are becoming increasingly more important and this trend cannot be ignored *(Table 1)*. Appropriate research efforts are thus needed to develop pragmatic control measures for them, including suitable supporting extension activities to implement them.

Other recent pests

In recent years, a few pests were observed to have become more important in a number of countries, independent of DBM suppression. The more prominent ones include the complex of leafminers comprising mainly *Liriomyza huidobrensis* (and other *Liriomyza* spp) and *Chromatomyia horticola* (Sivapragasam *et al.,* 1992) and *Spodoptera exigua* (Hussan, pers. comm) *(Table 1)*. The latter has recently been found to attack legumes and brassicas in Malaysia and is a key pest in Thailand. A wide range of insecticides are applied repeatedly against them. Like DBM, many of these chemicals will soon become ineffective, suggesting rapid development of resistance to the products. Also, in many places the problems have increased with more intense use of chemical

Table 1: Changing trends in importance of crucifer insect pests in the developing tropics of Asia

Relative importance	Insect pest	Remarks
Very important	**Hellula undalis* **Phyllotreta* spp. **Pieris* spp. particularly *P. rapae* and *P. brassicae* **Plutella xylostella*	Occur in abundance most of the time. Farmers spray regularly with many kinds of insecticides against them. Usually very difficult to control. Serious problem encountered with insecticide resistance development. [Note: In some cool highland areas where parasites (particularly *Diadegma semiclausum*) are providing good control, *P. xylostella* has become unimportant].
Moderately important	**Agrotis ypsilon* **Crocidolomia binotalis* **Spodoptera litura* *Aphids (mainly *Myzus persicae* and *Brevicoryne brassicae*	Present most of the time but peaks of abundance are sporadic. Farmers commonly spray insecticides to control them.
Becoming more important recently	*Leafminers (mainly *Liriomyza* spp. and *Chromatomyia* spp.) **Spodoptera exigua*	Believed these are spread around rapidly and widely by increasing movements of trade and people in recent times. Farmers spray heavily against them.
Becoming more important following effective suppression of *Plutella xylostella* by parasites	*Aphids **Crocidolomia binotalis*	These have been observed to increase recently in areas with effective IPM programmes against *P. xylostella*. Believed to be a case of pest succession where the niche previously occupied by *P. xylostella* is now taken over by these species.

insecticides, indicating that their buildups may be insecticide-induced.

These emerging pests are also believed to be introduced species. They probably have escaped quarantine and are spread around by the increased agricultural trade and other global activities of transnational travellers in recent years. Whatever the means of entry, these exotic pests can only be effectively managed if there also exist their complement of effective natural enemies. Numerous parasites of leafminers have been recorded (Waterhouse and Norris, 1987). However, these parasites are presently not fully exploited for leafminer problems in the Asian region. Efforts should be made to explore the possibility of utilising them, particularly after confirming the absence of useful indigenous natural enemies. Focusing on Pacific countries, Waterhouse and Norris (1987) suggested that because of the wide host range as well as the need to consider the wide range of cultivated plants at risk, it is probably desirable to introduce a wide range of parasites. They pointed out that no harm will result from this and, if it is done, there seems to be good prospects for bringing about biological control, provided insecticide application for the control of other pests is selective or carefully restricted. Species of particular promise are *Chrysocharis parksi, Chrysonotomyia punctiventris, Diglyphus begini, D. intermedius* and *Ganaspidium hunteri.*

Besides introductions of effective parasites, there is also a need for more vigilant enforcement of quarantine measures to prevent further spread of these pests into those countries where they are still not present.

Trends in pest control

Growing list of negative impacts of pesticides

Because many insects continue to persist as major pests leading to many undesirable problems from *pesticide treadmills*, the growing negative impacts of pesticides have become an increasing concern. For example, where DBM remains a serious problem, as is the case in most parts of the developing tropics, many of the undesirable impacts of pesticides are now widespread. In many countries, the problems continue to escalate, especially where DBM has persisted and where the pesticide industry is very active and numerous kinds of insecticides are easily obtained. However, for some areas in a few countries where DBM is successfully controlled by parasites (e.g., Indonesia, Philippines, Taiwan), there have been reports of drastic reductions in pesticide inputs in those areas (Ooi *et al.* 1992; Talekar, 1992; AVRDC, 1995; IIBC, 1996).

The negative impacts and the implications arising from excessive use of pesticides on vegetables are numerous and now well known as shown in Appendix 1. They include the effects on pest ecology, effects on domestic species, wildlife and other living organisms, reduction and loss in biodiversity, pesticides in food and food chain, contamination of the environment, impacts on human, and their hormonal effects. These negative impacts may escalate as pesticide use in the region continue to increase (RENPAP, 1994).

Increasing resistance to Bacillus thuringiensis by DBM

For a long time it was assumed that insects, including DBM, would not become resistant to *Bacillus thuringiensis* (Bt) because it is a biological agent. An experimental study by Devriendt and Martouret (1976) initially lent support to this. However, as field populations of DBM were repeatedly exposed to commercial Bt sprays in Hawaii, high levels of resistance appeared, nullifying the pathogen's usefulness (Tabashnik *et al.*, 1992). There are now signs that resistance to the Bt has also developed in various parts of the world where Bt is used fairly extensively (Shelton and Wyman, 1992; Hama 1992; Syed, 1992). However, the cases of resistance reported appear to be mainly against the HD-1 isolate of the *kurstaki* serotype and to some extent the *aizawai* serotype (Wright *et al*., 1996). There seems also to be some cross-resistance between both the serotypes (Tabashnik *et al*., 1992).

Recent research has suggested that resistance to Bt could evolve much faster than previously anticipated. Numerous strains of the bacterium produce many different Bt toxins. It has been assumed that each would evolve independently and the process complex and long. But it is now confirmed that a single and common recessive gene can confer resistance to four different Bt toxins (Cry1Aa, Cry1Ab, Cry1Ac and Cry1F). Crosses between a field population that had been heavily exposed to Bt toxins and a susceptible laboratory population produced offsprings which not only survived exposure to just one toxin but also developed resistance to all four, suggesting that the resistance may have been the consequence of a single gene mutation. In the study, 21% of individuals in the susceptible laboratory population were heterozygous for the multiple resistance gene as compared with an expected frequency of 1 in 10 000.

That resistance to Bt could evolve much faster than previously anticipated has many implications. The most critical is whether resistance management strategies can practically be developed for effective implementation by small crucifer farmers, and in time, before the valuable resource of Bt toxins in pest control is lost.

Efforts in alternative pest management

Largely because of the negative impacts of pesticides and increasing difficulties encountered in controlling many vegetable pests, much efforts have in recent years been devoted to find alternative approach in dealing with them. Different strategies are adopted by different interest groups. At the farm level, some farmers are pioneering into cultivation under large plastic rain shelters or net houses. Researchers, governments and donors are focusing into integrated pest management (IPM) research. In IPM implementation, extension workers have explored

several methods. More recently, the non-formal, bottom-up and self-discovery approach of the Farmers Field School (FFS) has been receiving increasing support, both by some governments, donors and NGOs. A third approach is pursued mainly by other environmental concerned or/and health conscious groups, comprising largely those practising organic or ecological farming.

Cultivation under protected environment (netted structures)

In parts of some countries in the Asian region (e.g., China, Philippines, Malaysia, Thailand, Vietnam), particularly in areas where strong winds are not a recurring problem, cultivation of crucifers and other vegetable crops under protected environment is being practised. Essentially, two kinds of netted structures are used; those that are covered completely with a net (*ca* 256 mesh) and those with nets on the side and a plastic sheet on top. One major reason for such a practice is to exclude insect pests, hence avoiding or minimising the use of chemical insecticides. Other advantages include avoiding damage from direct impact of rain, wash-off of applied chemicals against diseases, soil spillage onto crops, better fertilizer use, and less weed problems.

Although the netted structures afforded some protection, many insect pest problems could not be excluded as was found in Malaysia (Loke *et al.,* 1996). The major insect problems as indicated by respondent farmers in a survey were, in order of importance, *P. xylostella, H. undalis, S. litura, A. ypsilon* and flea beetles (*Phyllotreta* spp). Sometimes, aphids posed a problem. Initially, the problems were usually rare, but they would normally build up from the third planting onwards. Like insects, disease problems were also generally less within the netted structures than outside.

A wide range of insecticides continued to be used, mostly Bt, cypermethrin and methamidophos. On average, about 50% of the growers sprayed 2–5 times per crop in the netted structures compared to 5–10 times outside. They used 10–70% (av: 41%) less insecticides, although the concentrations used were the same. A similar situation was also found for the case of disease control where fungicides were substantially reduced. Normally, growers would mix insecticides with fungicides and foliar fertilizers in a single spray operation.

An IPM programme has been developed and evaluated (Loke *et al.,* 1996). Although preliminary results have shown it to be promising, further refinements are needed to improve its overall effectiveness and practical applications. Some measures of success have also been reported in Thailand (Vattanatangum, 1990).

Emphasis on IPM

In principle, IPM attempts to integrate the available pest control methods to achieve a farmer's most effective, economical, and sustainable combination for a particular local situation. Emphasis is placed on biological control, plant resistance, cultural control, and other non-polluting methods. Pesticides are used only when necessary as a last resort and only when cost/benefit analyses show that their use is truly justifiable and acceptable alternatives are lacking. There are now many IPM success stories reported for several crops in the region, including vegetables (Hansen, 1987; Tait and Napompeth, 1987; Teng and Heong, 1988; Talekar, 1992; Ooi *et al. 1992;* ADB, 1994). In addition, there exist numerous experimental efforts and successes in many parts of the world that need further verification on a larger scale at the farm level (Wiebers 1991).

To date, the successful IPM programmes have produced many benefits (Hansen, 1987; Tait and Napompeth, 1987; Teng and Heong, 1988; Talekar, 1992; Ooi *et al., 1992;* ADB, 1994). These include: (1) lower production costs (at farm level) compared with the conventional pest control method with its high inputs of pesticides, (2) enormous savings for governments from pesticide imports and reduced subsidies for pesticide use, (3) reduced environmental pollution, particularly improvement of soil and water quality, (4) reduced farmer and consumer risks from pesticide poisoning and related hazards, and (5) ecological sustainability by conserving natural enemy species, biodiversity and genetic diversity. At a more general level, the stability that IPM provides to agricultural production enhances political stability in a country where agriculture is a dominant sector of the economy. In the rural areas, it is important in developing local self-reliance through farmer empowerment (Kenmore *et al.*, 1994). Thus, IPM can achieve broad and long-lasting socioeconomic benefits far beyond plant protection activities. Many of these benefits are well illustrated by the example of vegetable IPM in the Cordillera highlands of the Philippines (IIBC, 1996). In this case, the parasite, *D. semiclausum*, reduced DBM to a level that was insignificant economically. Pesticide use by FFS farmers were reduced by 80%, including a big shift towards using only less toxic insecticides where needed. Fungicides alone were cut by 50–70% and fertilizers also reduced. The economic benefits included bigger cabbages and higher harvests because of improved crop husbandry practices, as well as, savings in production costs amounting to an average of 37%. Farmers who adopted IPM practices reported a return on investment of >400%, compared to 250% for those who did not. The reduced spraying also led to a noticeable increase in a wide variety of beneficial arthropods. The prerequisites for success were a proven IPM programme, a team of skilled FFS trainers, trained extension staff and adequate support for the season-long weekly sessions.

In general, IPM is a sustainable system and has many benefits, although the impacts may vary with different programmes. It can, and should, replace the current conventional agriculture which relies solely on intensive agrochemical inputs that have caused much unwanted problems. Many individuals and

organisations have recognised this, including many national governments, donors and NGOs. Consequently, there is now seen a stronger movement towards IPM in many of the countries in the region. Donor support and NGOs' activities in vegetable IPM are also more evident, as may be illustrated by some samples of recent programmes in vegetable IPM, *viz*: ADB-AVRDC support programmes of AVNET, SAVNET and CLVNET which include Bangladesh, Cambodia, India, Indonesia, Laos, Malaysia, Pakistan, Philippines, Sri Lanka, Thailand, and Vietnam; World Bank loan support programme for the Agricultural Rehabilitation Project in Vietnam; FAO Inter-country Programme for IPM in Vegetables covering Bangladesh, Laos, Philippines and Vietnam; ACIAR-assisted programme for vegetable IPM in China, ADB-IIBC Project for Highland Vegetables in Cordillera, Philippines; German-support Bread for the World programme in Vietnam; CARE programme in Bangladesh, and World Education in Indonesia. Because vegetables are important food crops and massive amounts of pesticides are rampantly used in their cultivation, it is envisaged that IPM programmes for them would continue to receive strong support. These will have important role in alleviating many of the problems now experienced with vegetable cultivation in the region.

Growing interest in ecological farming and unorthodox practices

In recent years, more and more interest is seen for food production and security to be viewed in the context of a healthier, environmental friendly, and sustainable agriculture, and need for investment in low external input and non-chemical alternatives. Many sustainable agriculture initiatives and approaches based on these principles have been pioneered and pursued, albeit by small concerned groups, comprising mainly those practising organic or ecological farming.

In practice, numerous models exist and are advocated. For the most part the farms exclude synthetic chemical inputs (pesticides and fertilizers) and approach a "closed fertility system" management. Examples of the different "schools" of organic/ ecological agriculture include biodynamic agriculture, permaculture, farming with effective microorganisms (EM), ecological/natural farming, and indigenous/ traditional agriculture (Tompkins and Bird, 1973; Tebecis, 1982; Fukuoka, 1985; Mollison, 1988; Higa, 1989; Parr and Hornick, 1989; Reijntjes *et al.*, 1992; Lampkin and Padel, 1994; Ong, 1994; Perlas III, 1994; Redfield, 1994). It relies on the science of ecology and respect the wisdom and practices of indigenous farmers.

Among these practitioners, some "schools" believe that most current farming endeavours, even including many organic farmers, have little understanding of life because most believe the farming processes to be purely chemical. Affirming that purely chemical and materialistic explanations of life have collapsed, such "schools", however, are pioneering a "second scientific revolution". The new science sees nature as alive and ensouled, and spirit as active in nature and the universe. It incorporates studies on non-material and spiritual properties of life and expands the meaning of ecology to include exploring and working with divine and cosmic forces that nurture the quality and nutritional value of food crops. Examples of such "schools" include Yoko Farming (Tebecis, 1982), biodynamic farming (Perlas III, 1994), and others (Tompkins and Bird, 1973; Redfield, 1994).

Presently, practioners of organic/ecological agriculture, including those of unorthodox practices, comprise only a small minority of the farming communities. But the numbers appear to be growing steadily, particularly with increasing demand by consumers for organic food free of chemical pesticides. Despite the lack of governmental support for such produce, many small and independent groups have emerged in the Asian countries (e.g., India, Japan, Malaysia, Philippines, South Korea, Thailand) to produce and market them. Initially, many were concerned with organic vegetables and fruits, but expanding into others later. In some of these countries, commercial companies, stores and special sections in some supermarkets, have even been set up in recent years to trade in such products. A few have labels to identify such special eco-products. Guided by this trend, it appears that the organic agriculture industry will continue to grow and expand further in the future, particularly as consumers are becoming more affluent and health conscious due to more and more countries in the region becoming industrialised with improved standard of living.

Related issues, concerns, and what may be done

Some of the above trends observed in crucifer cultivation are truly a cause for concern, in particular, the large number of persistent pests and the heavy reliance on chemicals for their control. That these problems have persisted for a long time, with some becoming even more acute, clearly reflect the weakness of current approaches in dealing with them. Besides, a few new ones have also emerged. All these, along with the other trends noted, have generated new concerns, additional issues and challenges, and have demanded a review of present practices in crucifer cultivation, particularly the pest management strategies and approach. Aspects demanding immediate consideration include various aspects of research, extension and also those relating to policy support.

Research

Ecological and holistic approach in research

With some exceptions, much of the research on crucifer pests until now lacks an ecological and a holistic approach. Each pest is usually considered by itself. Research on control also tends to focus on short term measures, predominantly on chemical pesticides. Hence, researchers and extension workers feel confident only in recommending pesticides. Consequently, farmers know only pesticides as a

means to deal with pests. This situation is further aggravated by the pesticide industry which is able to push chemical pest control hard, mainly because few alternatives can be offered by researchers and the extension workers. Under such conditions, it is not difficult to understand why farmers have become 'trapped' in the *pesticide treadmills* and there are now many undesirable problems of pesticides.

In nature, each crucifer pest does not occur in isolation by itself. Usually, several different pests may occur together with some overlaps, although each may sometimes peak at different stage of the crop. Some may also concentrate in different parts of the plant. As such, a particular control measure made against any one pest will tend also to affect others, except in the use of specific agents such as parasites (e.g., *D. semiclausum* targeting only DBM). Applications of Bt against DBM can however also affect other lepidopteran pests on the crop, e.g., *H. undalis*. Likewise, spraying chemical insecticides against other pests may also adversely affect any successful on-going IPM programmes where DBM is kept under control by its parasites. As such, research must therefore consider the overall pest complex on the crucifer crop and should aim to deal with them together. A holistic approach is necessary, taking into consideration both the pest-natural enemy ecology and the crop agroecosystems. The latter is important since crucifers are often planted along or in rotation with other crops, some of which also having a number of common pests. A truly holistic approach, however, should also include diseases, weeds, and other aspects which normally are a part and the process in crucifer production. The strategy, in practice, should be geared towards integrated crop management (ICM).

Research to address farmers' needs

Although research has contributed much to the advancement of agricultural science, it has for too long also been done with focus for research sake, often in the scientist's own pet area and to satisfy his/her academic curiosity. Usually, insufficient attention is given to address the real problems of farmers; consequently many pest problems have remained unsolved. This is accentuated by the research approach being normally top-down and lacking accurate or direct feedbacks from farmers of their problems.

In recent years, there is increasing appreciation in the weakness of such a research approach. Consequently, some scientists are initiating research efforts in a bottom-up manner by working closely with farmers and undertaking research which matches the farmers' needs. Some of these have been effectively achieved through researchers-extensionists-farmers cooperative activities as exemplified by the farmer participatory action research (PAR) activities in FFS. Because of their efficiencies, PAR should continue to be given emphasis and expanded. Also, agricultural scientists who do not undertake PAR should be mindful of their responsibilities in helping farmers and to make more effort in reorienting their research approach towards achieving this objective. They should ensure that their research is aligned with the needs of farmers.

More efforts in biological control

Many crucifers now widely cultivated in the developing Asian countries are exotic vegetables. So also are many of their pests. The latter are posing serious problems because the natural enemies which keep them under natural control in their original homelands are absent. Where these have been successfully introduced, as in the case of DBM parasites, the pest has become unimportant in those areas where there are no or little use of chemical insecticides, whereby the parasites can exert their full impacts (e.g., in Australia, Indonesia, New Zealand, Malaysia, Philippines, Taiwan).

Currently, many of the pests have remained important because few natural enemies appear to exist. Where some indigenous ones do occur, their impacts have normally been inadequate. Consequently, many farmers have continued to resort to relying heavily on chemical insecticides. The successes with introduced parasites for the control of DBM and the persistent failures in controlling many other crucifer pests by conventional means should provide a good lesson on the benefits of classical biological control and in encouraging similar attempts of such an ecological approach to deal with most of the remaining important crucifer pests.

The present inability to satisfactorily handle many of the crucifer pests is a clear reflection of the lack of ecological and biological control efforts towards managing the current pest problems. This is especially so since biological control has been the cornerstone of many successful IPM programmes. Future strategies should therefore include a more concerted effort to search for and import appropriate natural enemies against the major pests now afflicting crucifer vegetables in the region. Incorporating good biological control agents will help in providing the basis for other control tactics to build on and also paving towards the formulation of a good IPM programme. Also, these successes can greatly enhance FFS activites, without which, the benefits from FFS may be less significant. Likewise, FFS activities should be encouraged to educate farmers on the benefits of biological control agents so as to gain their full cooperation in ensuring and expediting the establishment of these beneficial agents. Many attempts at classical biological control in the past have failed because farmers did not clearly understand the efforts. They continued to spray, thereby creating conditions which are not conducive for the introduced beneficial agents.

Research into alternative control methods

Although there are research efforts on alternative control methods (biological control, agronomic and cultural techniques, planting systems, etc) and ecological studies, these are relatively limited. Consequently, undertanding of these aspects in relation to crucifer cultivation is still very poor. In contrast,

much exist on the use of chemical insecticides; hence, recommendations focusing on chemical control are aplenty. Consequently, we can expect to continue to immerse in the unwanted problems of pesticide use, including facing persistent pest problems caused by *pesticide threadmills*.

Under such a situation, what choice then do farmers have? Little, but continue to spray repeatedly! Unless recommendations on alternatives can be offered, their approach of "chemical farming" can never change. Also, unless there are substantially more research efforts devoted into ecological studies and alternative methods, sound recommendations on suitable alternative control cannot be developed. It is therefore clear that scientists must reorientate themselves towards an ecological approach and reallocate more efforts to develop and recommend pest management measures alternative to chemical pesticides. This is crucial if the present scenario of conventional farming practices is to give way to a more ecological and environmentally-friendly farming system, such as IPM and other forms of ecological/ organic agriculture.

Hidden costs of pesticides

Although much evidence exist on the unwanted effects of many pesticides, there is little effort made by way of relating them to costs, e.g., costs to human health, environment, natural ecology, etc. Such data are unavailable in most parts of the world. However, in the United States, Pimentel *et al.*, (1993) have computed the overall environmental and social costs for agriculture. The total estimated annual costs amounted to US$ 8 billion. Even this is believed to be underestimated (Pimentel *et al*., 1993).

In the developing Asian countries, the environmental and social costs can be very high, particularly when the negative impacts of agrochemicals are increasingly being reported. Presently, because such data are lacking, much arguments exist about their hidden costs. There is therefore an urgent need to gather the much-needed information. Since massive amount of pesticides are normally used in vegetable cultivation, in particular crucifers, initial studies made in this environment would appear apt. The data generated could help support and promote the need for alternative control strategies.

Extension and IPM implementation

Although extension is aimed at transferring useful research findings for implementation by farmers, generally little of this happens in practice. This is largely due to poor communication or cooperation between researchers and extension workers. Also, it is partly because these separate functions are handled by different agencies. Often, another contributing factor is due to the research findings being not practicable or are of little relevance to the needs of farmers. Hence, farmers do not adopt them. For effective extension, therefore, the basic requisites must include a close working relationship between researchers and extension workers plus relevance of the research to farmers' needs. Even if these conditions are fulfilled, the extension approach must still be appropriate and effective.

Experiences of IPM extension through FFS have shown such an approach to be highly effective. Although most studies are in rice, there are increasingly more and more in vegetables (e.g., in Bangladesh, India, Indonesia, Philippines, Vietnam). What the FFS entails and the processes involved have been lucidly described by Kenmore *et al* (1994). Accordingly, an FFS is usually attended by about 25 farmers from the same village or the same area. It is season-long. Once a week an IPM facilitator visits the FFS to help farmers conduct field experiments to understand IPM.

In the FFS, farmers compare and study sprayed and unsprayed fields and conduct agroecosystem analysis by making drawings that illustrate the condition of the crop plant in the study field, the population of herbivores and key natural enemies, and the field conditions. Based on the findings, farmers make decisions on whether to carry out any interventions. This ability to make a decision is helped by carrying out simple experiments to understand the hazards of chemical pesticides, particularly insecticides, on health and natural enemies. Experiments on impact of insecticides on natural enemies and others comparing field cages *with* and *without* natural enemies help farmers become aware of the risks involved in using insecticides. In addition, farmers also learn about the number of prey eaten by each natural enemy individual, food preference, and how the natural enemy species survive when no herbivores are available. Exercises and experiments are constantly updated through new research information from national and international institutions. In this way, FFS ensures new research information reaches farmers quickly.

Basically, FFS are conducted for the purpose of helping farmers to master and apply IPM field ecology management skills. Problems are seen as challenges, not constraints. The IPM programme teaches principles. It does not promote packages which tend to discourage learning and increase the dependence of farmers on central planners. All participatory research carried out are responsive to field needs, quite unlike most conventional research programmes which drive the extension or education programme that the research should actually be serving.

To achieve this level of educating farmers, a cadre of good facilitators or extension workers is first trained. These facilitators undergo a season-long training-of-trainers programme where they learn to grow the crop and carry out experiments to learn about nonformal education, and develop a curriculum for FFS. Presently, this human resource is rather limited. Consequently, extension has been weak in many of the countries. Besides other infrastructural needs (e.g., vehicles, other materials required for field schools,

etc), more support must be given towards developing the required human resource. Unless sufficient human resource is available, field implementation of pest management programmes on a large scale will be difficult to achieve and will proceed only very slowly.

Policy support

A policy environment which discourages the indiscriminate use of pesticides is important for the development of alternative pest control strategies. This may even be a prerequisite in initiating programmes in alternative agriculture (e.g., IPM, organic agriculture). Government economic policies can often undermine a scientifically well-designed programme on alternative agriculture. For example, government technology "packages" that include substantial use of pesticides, or government subsidies for pesticides, will encourage pesticide overuse and reduce the economic incentives to use alternative pest control methods. Furthermore, in some countries, legislation concerning the regulation of pesticide imports, production, distribution, and use may be either absent of ineffective.

On the other hand, some policies may favour alternative agriculture. For example, media publicity on the dangers of pesticides, including residues in food, will encourage its demand. Since policy issues can have a great influence on the development and implementation of alternative agriculture, those involved must be aware of such issues and be able to create conditions that are conducive to it. They include key policy makers in the national governments and their scientists and extension workers, leaders and scientists in international organisations, NGOs and the donors. In general, the following policies are conducive to alternative agriculture and should be encourged.

* Reduce or eliminate subsidies for pesticides and other credit programmes that distort economic comparisions with non-pesticide alternatives.
* Adopt alternative agriculture as a significant part of national agricultural policy.
* Reorient national research, extension and agricultural education programmes towards non-pesticide practices.
* Earmark specific and adequate funds to ensure that national agencies have the resource to carry out the national agricultural policy on alternative agriculture.
* Promote the phased implementation of alternative agriculture to ensure that sufficient time is given to develop and demonstrate non-pesticide practices that are reliable and sustainable.
* Establish a national monitoring system for pesticide residues and the health impacts of pesticide use, i.e, hidden costs, and publicise the findings.

In recent years, new pests have also emerged, apparently resulting from entry into a country with movements of trade or people. To prevent or reduce further spread, there is therefore a policy need to institute and enforce more stringently the existing quarantine control measures. This requirement will become even more important when the General Agreements on Tariff and Trade (GATT, now W.T.O.) becomes widely and fully operational in the future whereupon trade liberalisation will increase substantially.

Discussion

The overall scenario of crucifer insect pest problems and their trends, related issues, and management strategies are summarised in *Figure 1*. Most of the different aspects not only are interrelated but are also influencing one another. Though all are important and need simultaneous attention, some require more effort and focused actions because of their priorities. The key area, wherein lies the root cause of the problems, is a lack in orientation and focus on an ecological and non-pesticidal approach to managing the pest problems. Even until now, negligible resources have been devoted to this area. In contrast, there has been enormous inputs into promoting the pesticide approach only. Only in recent years has there been some reconsideration because of the mounting unwanted side effects encountered in the use of pesticides. Even so, the reversal process has remained largely academic. Most proposals on alternatives agreed upon merely stay rooted in repeated debates and discussion tables, with little actually acted out. Except for a few governments, most are either unable to perceive the need for reversal (e.g., to ecological/organic agriculture, or IPM), or even if able to, are lacking in commitment to follow through. Even many donor agencies are suffering the same way. It is no wonder so many major pests of crucifers have persisted until today, not forgetting a few new ones adding on recently.

A reason commonly cited for the little effort put into ecological agriculture in the past is insufficient resources (funds, human, expertise, others). In reality, it is not so much a lack, but more of an incorrect orientation or disproportionate allocation of the existing resources. If one were to examine closely the resources allocated to pest management programmes (e.g., research, crop production activities, subsidies, promotional programmes, etc), it will at once be evident that disproportionately little has all along been devoted to activities promoting alternative agriculture. This can be clearly seen in practically all national government allocations in the region. Again, this is also true for those of many international organisations, including donor support programmes. Consequently, alternative and ecological/organic agriculture cannot be expected to grow otherwise, but only very slowly.

If national governments, international agencies and donor support programmes were to now have a policy to support more fully organic and other

alternative agriculture, and reallocate, say, even only 50% of their pest management and crop production resources towards the support of such non-pesticide programmes, it can be envisaged that much understanding and technologies would rapidly become available within the decade, or even a shorter period, by way of alternative agriculture, particularly in ecological/organic farming. Because of policy change, hence reorientation of resources (people, funding, infrastructure, incentives, etc), the research programmes, emphasis and focus will immediately be redirected to produce the technologies needed to develop, support and sustain alternative agriculture, including ecological/organic farming. In parallel, other undertakings to support the alternative agriculture economies would likewise receive emphasis and be developed. These would include appropriate government incentives (e.g., initial support subsidies), legislative regulatory protection (e.g., certification or eco-labelling of organic produce, or even IPM products with no detectable pesticide residues), and assistance in administrative and commercial infrastructural support (e.g., competitive commercialisation of natural enemies for pest control, easily available compost or organic fertilizers, etc). Unless such concerted efforts are directed towards developing an efficient distribution and marketing network for a viable alternative agriculture industry, it would take a very long time, or probably even impossible, to overcome the current pesticide-based agriculture that has already gained a strong foothold in most national economies.

Should the present trends persist, which are likely if there is no firm policy change towards alternative agriculture, it is probable that much of the pest and pesticide problems we now encounter with crucifer cultivation (or vegetables and other crops in general) will continue, or even escalate, well into the next millennium, proceeding in the same way of the past. We are now staring at the problems because we did not fully foresee the ecological implications and thus have failed to deal with them effectively over the past decades. But, failing to act quickly in reorienting towards the ecological approach now that we have comprehended its importance would merely mean we lack foresights, could not learn from past mistakes, or are irresponsible. If so, we therefore deserve to continue to inherit all the crucifer pest problems and others associated with pesticide-based control practices.

Judging by situations in the past, to look for a drastic change appears to hold little promise. However, there is a glimmer of hope in that several agricultural sectors have acknowledged the serious problems of pesticides and some have begun efforts to address them. For example, the Malaysian Agricultural Research & Development Institute (MARDI) has in recent years set up a unit on natural farming to develop organic agriculture. Many NGOs in the region also have such initiatives. Although there is increasing funding support towards IPM by donors, very few gave support to other forms of alternative agriculture, e.g., organic farming. These efforts, however, are relatively small in comparision with those activities which still use a lot of pesticides. At least IPM, has gained wide acceptance and is now actively being promoted in the region, including on crucifers. Although IPM still accepts a minimum amount of pesticide inputs where needed, it nevertheless can play crucial role towards reducing quite substantially the present pesticide use. It also will help slow down, but not curb, the uncaring onslaught of conventional agriculture.

Despite it being widely accepted, IPM development and implementation, particularly on a wide scale, have continued to face a number of constraints. Though many general attributes and requirements for IPM success are now known, these need to be properly addressed.

Firstly, it is important to destroy the myth that IPM is too complicated. Experience has shown that IPM can work well in developing countries and is within the reach of farmers who have received appropriate training and help in terms they can understand. For example, IPM in a number crops in many developing countries, including crucifers in Southeast Asia, has reduced pesticide applications 30–100% while yield stayed the same, or even increased. The net result may be a higher profit to the farmer.

Another important attribute is political commitment or governmental support to IPM. It can be disastrous if this is lacking. For example, a policy environment conducive to IPM will serve to discourage the use of pesticides. Adequate technical information and technologies are also important. These should meet the needs and capabilities of farmer groups. On research in IPM, it must involve extension workers and the farmers so as to ensure that cultural and social realities of farmers are included. The research must be field-oriented and geared at solving farmers' problems while considering appropriate risk management. For insect-based IPM, emphasis on biological control is crucial. Its absence, or disruption when present, is usually the main reason for a continuous insect problem. Introducing effective biological control agents is therefore a necessary first step if these are absent. Presently, most IPM still applies to single pest/crop. A holistic or ecosystem approach is necessary. Special consideration should also be given to human resource development. In particular, farmers' training must emphasise the hands-on and self-discovery approach because IPM is not something which is *done for farmers* but *done by farmers*. Funding is crucial, especially for implementation of selected IPM projects which have adequate support to strengthen farmers' training.

Because IPM still permits the use of some agropesticides, unlike ecological or pure organic agriculture, IPM can be seen as a first forward step away from the current "chemical farming" and moving towards a more stable and sustainable alternative agricultural system. Ensuring its adoption over a wide scale is thus crucial and could possibly pave the way for a full reversal to alternative agriculture eventually.

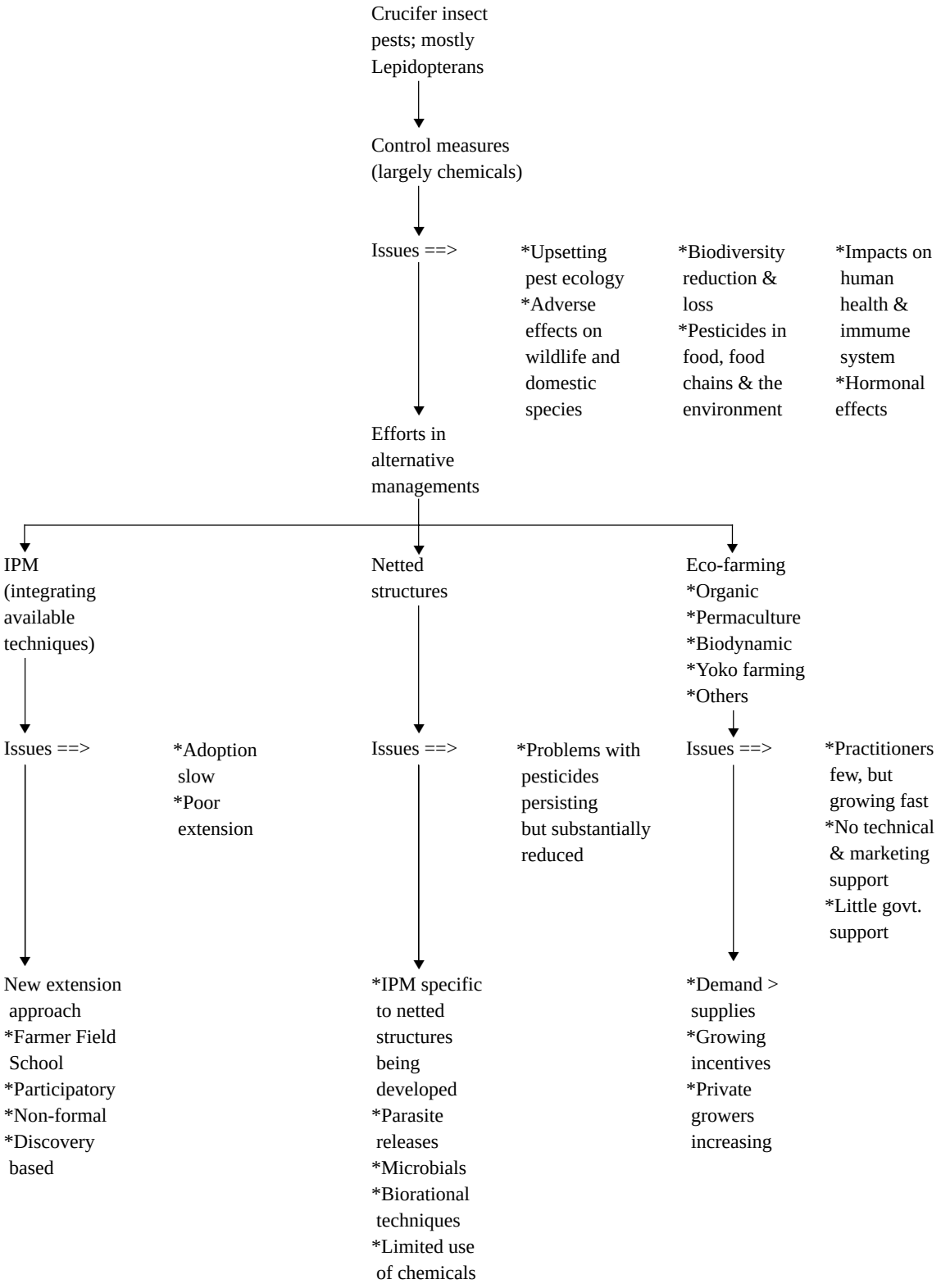


Figure 1: Scenario of crucifer insect pest problems and their related issues and management strategies.

Through this process, many mutual benefits with nature can be reestablished and gainfully harmonized. Every effort, therefore, should be made by all concerned to achieve it.

References

ADB (1994). *Handbook for Incorporation of Integrated Pest Management in Agriculture Projects*. Asian Development Bank, Manila. 125 pp.

AVRDC (1995). Proceedings of the AVNET II MidtermWorkshop. Asian Vegetable Research and Development Center. Shanhua, Tainan, Taiwan. Pub. No. 95–438. 328 pp.

CAB International (1994). *Using Biodiversity to Protect Biodiversity (Biological Control, Conservation and the Biodiversity Convention)*. CAB International, Wallingford, UK. 14 pp.

Castaneda, C.P. (1988). Pesticide poisoning studies and data collection in the Philippines, 311-314. *In*: Teng, P.S. and Heong, K.L. (eds). *Pesticide Management and Integrated Pest Management in Southeast Asia.* CICP, Maryland.

Devriendt, M. and Mortouret, D. (1976). Lack of resistance in the diamondback moth, *Plutella maculipennis* (Lep.: Hyponomeutidae) to *Bacillus thuringiensis* (in French, English summary). *Entomophaga* 21: 189–199.

Edwards, C.A. (1991). The impacts of pesticides on the environment, 13–46. *In*: Pimentel, D. and Lehman, H. (eds). *The Pesticide Question: Environment, Economics, and Ethics*. Chapman & Hall, New York and London. 441 pp.

FAO/WHO (1978). *Guide to Codex, Maximum Limits for Pesticide Residues.* CAC/PR 1. Rome, FAO.

Fukuoka, M. (1985). *The Natural Way of Farming: Theory and Practice of Green Philosophy.* Japan Publication, Inc., Tokyo & New York. 284 pp.

Hama, H. (1992). Insecticide resistance characteristics of the diamondback moth. *In:* Talekar, N.S. (ed.). Management of diamondback moth and other crucifers pests: proceedings of the Second International Workshop, Shanhua, Taiwan. pp:455–63

Hansen, M. (1987). *Escape from the Pesticide Treadmill: Alternatives to Pesticides in Developing Countries.* Organization of Consumers Unions and Pesticide Action Network. Consumers Union of United States, Inc. 185 pp.

Higa, T. (1989). Microorganisms will save the earth. Komo Newspaper (August 1), Japan.

IIBC (1996). Final TA Report: Integrated Pest Management for Highland Vegetables. Vol. 1. International Institute of Biological Control, Baguio City, Philippines. 291 pp.

Kenmore, P.E., Gallagher, K.D. and Ooi, P.A.C. (1994). Empowering farmers: Experiences with integrated pest management. *Entwicklung & Landlicher Raum,* 1/95, 27–28.

Kritalugsana, S. (1988). Pesticide poisoning studies and data collection in Thailand, 321–324. In: Teng, P.S. and Heong, K.L. (eds). *Pesticide Management and Integrated Pest Management in Southeast Asia.* CICP, Maryland.

Lampkin, N.H. and Padel, S. (eds). (1994). *The Economics of Organic Farming: An International Perspective.* CAB International, Wallingford, UK. 468 pp.

Lim, G.S. (1990). Overview of vegetable IPM in Asia. FAO Pl. Prot. Bull. 38: 73–87.

Lim, G.S. and Di, Y.B. (1990). (eds). *Status and Management of Major Vegetable Pests in the Asia-Pacific Region (With Special Focus Towards Integrated Pest Management).* Regional Office for Asia and the Pacific (RAPA) Publication, FAO: 1990/3. 126 pp.

Loke, W.H., Sivapragasam, A., Fauziah, I. and Md. Jusoh Mamat. (1996). Employing the IPM concept in managing insect pests of crucifers grown under netting, 51-63. In: Sivapragasam, A., Mah, S.Y., Ramli Mohd. Nor and Loke, W.H. (eds). *Collaborative Vegetable Research Program in Southeast Asia (AVNET - II; 1993–1996). Project Completion Report.* MARDI, Serdang, Malaysia.

Mollison, B. (1988). *Permaculture - A Designers' Manual.* Tagari Publication, Australia.

Mustamin, M. (1988). Health hazards due to the use of pesticides in Indonesia: data collection and surveys, 301–310. *In:* Teng, P.S. and Heong, K.L. (eds). *Pesticide Management and Integrated Pest Management in Southeast Asia.* CICP, Maryland.

Ong, K.W. (1994). Getting it together: an integrated multi-sector, multi-level platform for sustainable agriculture development. IFOAM Caucus Meeting, 11 December 1994, Christchurch, New Zealand. 20 pp. (mimeo).

Ong, S.H. (1990). Degradation of endosulfan and permethrin on sawi, *Brassica rapa* (L.) - implications on insect management: 55–61. *In*: Proc. 3rd Internatl. Conf. on Pl. Prot. in the Tropics, 20–23 March 1990, Genting Highlands. Malaysian Pl. Prot. Soc., Kuala Lumpur.

Ooi, P.A.C., Lim, G.S., Ho, T.H., Manalo, P.L. and Waage, J. (eds). (1992). *Integrated Pest Management in the Asia-Pacific Region.* CAB International, Wallingford, UK. 459 pp.

Parr, J.F. and Hornick, S.B. (1989). Recent developments in alternative agriculture in the United States. Paper presented at: The First Internatl. Conf. on Kyusei Nature Farming, 17–21 October 1989, Khon Kaen Univ., Khon Kaen, Thailand.

Perlas III, J.N. (1994). *Bio-Dynamic Vegetable Farming: A Training Manual.* Publ. J.N.Perlas III, Baguio City, Philippines. 78 pp.

Pimentel, D., Acquay, H., Biltonen, M., Rice, P., Silva, M., Nelson, J., Lipner, V., Giordano, S., Horowitz, A. and D'Amore, M. (1993). Assessment of environmental and economic impacts of pesticide use, 47-84. *In*: Pimentel, D. and Lehman, H. (eds). *The Pesticide Question: Environment, Economics, and Ethics*. Chapman & Hall, New York and London. 441 pp.

Ramasamy, S. and Nursiah, M.T.A. (1988). A survey of pesticide use and associated incidences of poisoning in Peninsular Malaysia. *J. Pl. Prot. in the Tropics*. 5: 1–9.

Redfield, J. (1994). *The Celestine Prophecy: An adventure.* Bantam Books, London/New York/Toronto/Sydney/ Auckland. 282 pp.

Reijntjes, C., Haverkort, B. and Waters-Bayer, A. (1992). *Farming for the Future.* The Macmillan Press Ltd., London, UK. 249 pp.

RENPAP (1992). UNIDO Regional Network on Pesticides for Asia and the Pacific (unpublished report).

Repetto, R. and Balilga, S. S. (1996). Pesticides and the immune system: The public health risks. World Resources Institute, Washington D.C. 5 pp.

Rola, A.C. and Pingali, P.L. (1993). *Pesticides, Rice Productivity, and Farmers' Health: An Economic Assessment.* IRRI (Los Banos) and World Resources Inst. (Washington). 100 pp.

Shelton, A.M. and Wyman, J.A. (1992). Insecticides resistance of diamondback moth in North America. *In:* Talekar, N.S. (ed.). Management of diamondback moth and other crucifers pests: proceedings of the Second International Workshop, Shanhua, Taiwan. pp:447–54.

Sivapragasam, A., A.R. Syed and W.H. Loke (1992). The leafminer, *Chromatomyia horticola (*Goureau) and its increasing concern to farmers in Cameron Highlands. *MAPPS Newsletter* 16(3): 19–20.

Solbrig, O.T. 1994. Biodiversity: an introduction:13-20. *In*: Solbrig, O.T., van Emden, H.M. and van Oordt, P.G.W.J. (eds). *Biodiversity and Global Change.* CAB International, Wallingford, UK. 227 pp.

Syed, A.R. (1992). Insecticide resistance in the diamondback moth in Malaysia: 437–442. *In:* Talekar, N.S. (ed). *Diamondback Moth and Other Crucifer Pests.* (Proc. 2nd Internatl. Workshop, 10–14 December 1990, Tainan, Taiwan). AVRDC, Taipei, Taiwan. 603 pp.

Tabashnik, B.E., Cushing , N.L., Finson, N., Johnson, M.W. (1992). Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). *J. Econ. Entomol*. **83**: 1671–1676.

Tait, J. and Napompeth, B. (eds). (1987). *Management of Pest and Insecticides: Farmers Perception and Practices.* Westview Press, London, UK.

Talekar, N.S. (ed). (1992). *Diamondback Moth and Other Crucifer Pests.* (Proc. 2nd Internatl. Workshop, 10–14 December 1990, Tainan, Taiwan). AVRDC, Taipei, Taiwan. 603 pp.

Tebecis, A.K. (1982). *Mahikari: Thank God for the Answers at Last.* L.H. Yoko Shuppan (Publishing) Co. Ltd., Tokyo, Japan. 462 pp.

Teng, P.S. and Heong, K.L. (1988). *Pesticide Management and Integrated Pest Management in Southeast Asia.* Consortium for Internatl. Crop Prot., Maryland, USA. 473 pp.

Tompkins, P. and Bird, C. (1973). *The Secret Life of Plants.* Harper & Row Publishers, Inc., New York. 402 pp.

Vattanatangum, A. (1990). Status and management of major vegetable pests in Thailand: 91–95. *In:* Lim, G.S. and Di, Y.B. (eds). *Status and Management of Major Vegetable Pests in the Asia-Pacific Region (With Special Focus Towards Integrated Pest Management).* Regional Office for Asia and the Pacific (RAPA) Publication, FAO: 1990/3. 126 pp.

Waterhouse, D. F. and Norris, K. R. (1987). *Biological Control: Pacific Prospects.* Inkata Press, Melbourne. 454 pp.

Wiebers, U. (1991). Agricultural technology and environmental safety: integrated pest management and pesticide regulation in developing Asia. Technical Department Asia Region, Agriculture Division, World Bank. 78 pp. (mimeographed report).

Wright, D.J., Iqbal, M., Granero, F. and Ferre, F. (1996). Resistance mechanisms to *Bacillus thuringiensis subsp. kurstaki* and *Bacillus thuringiensis subsp. aizawai* in a multi-resistant field population of diamondback moth from Serdang, Malaysia. Paper presented at the Third International Workshop on the Management of Diamondback Moth and Other Crucifer Pests, Kuala Lumpur, Malaysia.

Appendix 1. Negative impacts from excessive use of pesticides in agriculture (including crucifer vegetables)

1. Upsetting Pest Ecology: Insect outbreaks induced by insecticides may result because some chemicals can selectively kill the pest's natural enemies. Without the latter, and especially where the insecticide is insufficiently effective because the pest has become resistant, then pesticide-induced outbreaks can readily occur. Applying insecticides may therefore sometimes create rather than suppress a pest problem.

Another effect on pest ecology is that pests can readily develop resistance to the insecticides used against them. Today, this is proliferating in many parts of the world. A classic example is that of diamondback moth on crucifer vegetables in Southeast Asia where the moth has become resistant to almost all kinds of insecticides introduced into the market (Lim, 1990). In Hawaii (Tabashnik *et al.*, 1992) and elsewhere, it has even become resistant to the biological insecticide, *Bacillus thuringiensis*.

The effects of insecticide-induced outbreaks and insecticide resistance can combine to cause a phenomenon known as the *pesticide treadmill* characterised by an upward spiral of insecticide use, increasing input costs, and declining income as uncontrollable number of insects reduces yield. Today, *pesticide threadmills* have occurred on many crops, including crucifer vegetables (Hansen, 1987, Teng and Heong, 1988, Ooi *et al.*, 1992).

2. Adverse Effects on Domestic Species, Wildlife and other Living Organisms: The adverse impacts of pesticides have included numerous nontarget species, both domestic (usually pets, poultry and livestock) and wildlife (mostly earthworms, aquatic lives, honeybees and beneficial arthropods, and birds). In aquatic systems, they are mostly invertebrate species (such as crustaceans) and fish which may also be used as food.

Soil-inhabiting species such as springtails (Collembola), pauropods, symphylids, millipedes, centipedes and other ground dwelling arthropod predators, and earthworms, are also severely affected (Edward, 1991). They have benefitting role in crop production. Mites which occur abundantly are badly affected by acaricides, some insecticides and soil-fumigants, in particular the more active predatory species.

Effects on microorganisms are more complex and less understood because some can utilize pesticides as food sources or are involved in complex food chains. In general, any effect is relatively transient; the populations usually recovering in 2–8 weeks.

3. Reduction and Loss in Biodiversity: Agricultural intensification can adversely affect biological and genetic biodiversity. Heavy insecticidal use kills both insect pests and other organisms. A field freshly treated with wide-spectrum insecticides usually contains very little biological activity while even an adjacent untreated one will retain its faunal richness, both in numbers and diversity. In treated fields, biodiversity will gradually increase only if there in no further treatment. Otherwise, biological life will remain relatively very poor.

Natural enemy species used in biological pest control constitute a large component of the world's biodiversity and are valuable to sustainable agriculture. They can often replace pesticide inputs. Topping the food chains, they play important role in regulating the balance of their preys to ensure co-existence of species by allowing none to become too abundant (CABI, 1994). Biodiversity, a property of living systems (Solbrig, 1994), is a characteristic of nature; thus, destroying biodiversity is destroying nature.

4. Pesticide Contaminations in Food and Food Chain: Pesticide residues are found in many market produce, particularly fresh fruits and vegetables (Lim, 1990). For example, tomatoes obtained from several local markets had residues of dithiocarbamate fungicides ranging from 0.21 mg/kg CS_2 to 15.8 mg/kg CS_2 . About 65% of these contained residues in excess of the maximum residues limit of 3 mg/kg CS_2 set by FAO/WHO (1978). When permethrin was applied on *Brassica rapa* at 0.04%, the total residues may reach 10.31 ug/g. In the case of endosulfan (applied at 0.5%), the level of total residues reached was 184.28 ug/g. Ong (1990) recommended against using endosulfan on short term crops where 14-21 days waiting period cannot be practised.

High pesticide residues were also found in many market vegetables in Indonesia, the Philippines and Thailand. The widespread occurrence suggests that many currently unsurveyed produce in the region may also contain excessive residues. This has far reaching implications in trade between countries because of rejections by importing countries of the contaminated produce.

5. Contaminating the Environment: Enormous quantities of pesticides are used on crucifer vegetables and are increasing. The full degradation pathways or ultimate fate of many in the field are still not fully known. Many pesticides do not reach their targets but instead end up in the crops, other vegetations, animals, soils, or water. Persistent residues usually end up in soils or aquatic sediments in water bodies.

6. Impacts on Human: Pesticides also harm human health. Both acute and chronic poisonings have long been reported, although deaths may not frequently result. For example, 28.1% of 153 vegetable growers surveyed in Malaysia suffered poisoning symptoms, including headache, dizziness, nausea, and general fatigue soon after spraying operations (Ramasamy and Nursiah, 1988). Sometimes, skin rash and dermatitis are evident. Often, mild poisoning symptoms may be ignored because they are taken as signs of working too hard or due to influenza as noted in Indonesia (Mustamin, 1988). In Thailand, there were 4 046 poisoning cases in 1985 (Kritalugsana, 1988) and 824 cases in the Philippines in 1984 (Castaneda, 1988). However, not all of these are due to accidental or occupational poisonings. Substantial numbers are because of suicides.

On human health impact, the costs can be quite significant as found for rice. For example, comparative studies made in the Philippines on health status of farmers exposed to pesticides with those unexposed showed that chronic impairment of health was associated with prolonged exposure (Rola and Pingali, 1993). Farmer health costs increased by 0.74% for every 1% increase in insecticide dose. The health impairments included eye, skin, lung, cardiovascular, and neurological diseases.

More recently, there are concerns about public health risks from pesticide-induced suppression of immune system (Repetto and Baliga, 1996). Many tests revealed that a variety of organochlorine, organophosphate, carbamate, and metallic pesticides are immunotoxic and can alter the immune system's normal structure, disregulate and disturb immune responses, and reduce the resistance of exposed animals to antigens and infectious agents. That pesticides are immunosuppresive in humans are increasingly being suggested by both indirect and direct evidence.

7. Hormonal Effect: Recently, some pesticides with weak potential to imitate the female estrogen hormone on their own were found to become hundreds of times more potent when they are combined. For example, test of four pesticides (chlordane, dieldrin, endosulfan and toxaphene which are weakly estrogenic alone) on genetically engineering yeast cells showed the estrogenic effect was greatly enhanced when they are paired. In particular, potency in the pair of endosulfan and dieldrin increased substantially by 160-1 600 times.

The findings suggest that exposure to mixes of chemicals routinely found in the environment could be posing a much greater risk than suspected and that normal government screening of pesticides may be inadequately protecting the public from reproductive ailments and declining fertility. Evidence that "gender-warping" chemicals boost estrogen or block testosterone and damage sex organs has emerged only in recent years and the evidence is mounting steadily. Tests have shown that several manmade chemicals can damage even at low levels the sexual development of a fetus. While the adult is unaffected by the exposure, the damage is passed to the next generation through the womb. Among wildlife, many animals including alligators in Lake Apopka (Florida), birds in the Great Lakes (Michigan) and otters in Columbia River are born with super-estrogen levels, small penises or malformed testes. All these are suspected to be linked to pesticides and other industrial chemicals.

Prospects for novel approaches towards management of the diamondback moth

A. M. Shelton[1], C. J. Perez[1], J. D. Tang[1] and J. D. Vandenberg[2]

[1]Department of Entomology, NYSAES/Cornell University, Geneva, NY 14456
[2]USDA/ARS, Ithaca, NY 14853

Abstract

Primary reliance on synthetic insecticides for control of diamondback moth has usually failed because of insecticide resistance and/or reduction of natural enemies by insecticides. Insecticide resistance has also been more recently documented with biological insecticides such as *Bacillus thuringiensis* and some growth regulators. Insecticide resistance management programs must be implemented and new alternative management strategies must be developed. Our laboratory has documented high levels of resistance to the major insecticides, including *Bacillus thuringiensis* subsp. *kurstaki*, used against diamondback moth and proposed discriminating doses to assess potential field performance. Additionally, we have tested resistance management strategies for use of *Bacillus thuringiensis* when applied as a foliar spray and when incorporated into plants. Such strategies include both refuge size and placement. In laboratory, greenhouse and field studies we have promising results for managing diamondback moth using foliar sprays of a commercial formulation of *Beauveria bassiana*. However, we have had less promising results controlling diamondback moth through the presently available technology for mating disruption using pheromones.

Key words: Diamondback moth, *Plutella xylostella*, resistance management strategies

Introduction

In the past 40 years the diamondback moth, *Plutella xylostella* (L.) has become the most destructive insect pest of crucifers worldwide, with an estimated annual cost of managing it of US$1 billion. The reasons for its continued success against our present approaches are several, including its high reproduction potential, the disruption or lack of natural enemies, and its ability to become resistant to a wide range of toxins and growth regulators. A review of the biology, ecology and management of diamondback moth (Talekar and Shelton, 1993) provides a more complete perspective of its present status and potential management opportunities. The purpose of this paper is to discuss some of the more recent management approaches which are being undertaken.

Tactics

Biological Control

Microbial Control

Bacillus thuringiensis: *Bacillus thuringiensis* is not the most toxic material which can be used against *P. xylostella* but it has the advantage of being non-toxic to natural enemies and therefore will not be disruptive to the system. The effectiveness of *B. thuringiensis* can be enhanced through the use of application methods which provide a more uniform coverage of the plants (Perez *et al.*, 1995). In fact, using improved application technology may be equivalent to a 'low dose' resistance management strategy since it will reduce the number of applications per season while providing adequate control. In Malaysia the use of *B. thuringiensis*, in combination with parasitoids, is the cornerstone of the IPM program and spraying *B. thuringiensis* is recommended when a tentative economic threshold of 5 larvae per 10 plants is exceeded (Loke *et al.*, 1992). The *B. thuringiensis* species which is used most commonly is *B. thuringiensis* subsp. *kurstaki*. Resistance to the d-endotoxins of *B. thuringiensis* subsp. *kurstaki* has been documented in *P. xylostella* in the continental USA (Shelton *et al.*, 1993), Hawaii (Tabashnik *et al.*, 1990), Malaysia (Syed, 1992) and Central America (Perez and Shelton, 1996a). Failure of *B. thuringiensis* subsp. *kurstaki* in Florida, where field-developed resistance was >1 500-fold, was due to resistance to the CryIA protoxins (Tang *et al.*, 1996). More recently, low levels of resistance to the CryIC toxin contained in *B. thuringiensis* subsp. *aizawai* have been documented in Hawaii (Liu *et al.*, 1996) and Central America (Perez and Shelton, 1996a). Because of the importance of *B. thuringiensis* for the overall management of *P. xylostella*, it is imperative that resistance management strategies be developed before the problem becomes more widespread. As a first step in this, we have developed leaf-dip and diet-incorporated assays which can be used as a diagnostic concentration to differentiate *B. thuringiensis* subsp. *kurstaki* -resistant populations in the field (Perez and Shelton, 1996a; Perez *et al.*, 1996a). Especially, the diet-incorporated assay will allow growers and extensionists to rapidly assess problematic populations so that other tactics can be used, at least on a temporary basis.

Resistance management strategies for field sprayed commercial formulations of *B. thuringiensis* against *P. xylostella* in the field are desperately needed to ensure its long term durability. In the only field study to date (Perez *et al.*, 1996b), resistance management strategies were tested during the dry and rainy seasons of 1995 in Honduras. Over 5–6 generations of field selection with 16 applications, resistance to a commercial formulation of *B. thuringiensis* subsp. *kurstaki* significantly increased when a high dose strategy was used, regardless of the presence or absence of a 25% refuge, compared with a low dose strategy. Inclusion of a refuge in either a low or high dose strategy may reduce the proportion of marketable produce and thereby reduce adoption of this strategy. While resistance to *B. thuringiensis* subsp. *aizawai* did not increase substantially after field selection in our tests, it is likely that over a longer period resistance to the CryIA and the CryIC toxins would occur. The use of *B. thuringiensis* subsp. *aizawai* against *B. thuringiensis* subsp. *kurstaki*-resistant *P. xylostella* may maintain the resistance to individual toxins produced by both *B. thuringiensis* species (Tang *et al.*, 1995).

Will *B. thuringiensis* ever be a durable and highly effective product? Because of the generally poorer performance of *B. thuringiensis* compared with newer synthetic insecticides, the outlook may not be too hopeful. One way of making *B. thuringiensis* more effective is to have plants express the toxins, but there is concern about development of resistance with this strategy. Our laboratory is trying to address a proactive resistant management strategy for plants which are engineered to express foreign toxins. Transgenic crucifer plants expressing the CryIA(c) gene have been produced (Metz *et al.*, 1995) and these plants have expression levels which will kill susceptible larvae but which will not control resistant *P. xylostella*. In greenhouse studies we have been able to demonstrate the value of separate refuges for preserving genes for susceptibility (Tang and Shelton, unpublished) and these tactics are presently being tested in the field with very promising results.

Fungi

Fungi may prove to offer another major avenue for microbial control of *P. xylostella*. Several fungi have been isolated from *P. xylostella* (Humber, 1992) and natural epizootic of two Entomophthorales species in Asian populations of *P. xylostella* (Riethmacher and Kranz, 1994) have been described. Pell and her colleagues (Pell *et al.*, 1993a, b) have studied infections of *Zoophthora radicans* and Ibrahim and Low (1993) have shown the potential effectiveness of *B. bassiana* and *Paecilomyces fumosoroseus* in the field.

Vandenberg and his colleagues have been working to incorporate fungi into *P. xylostella* management schemes in the US and have developed appropriate laboratory assays. To date they have tested >55 isolates of *B. bassiana*, *Fusarium* sp., *Metarhizium anisopliae* and *Paecilomyces farinosus* (Vandenberg and Ramos, 1997) as well as determined the dose response and age- and temperature-related susceptibility of *P. xylostella* larvae to two *B. bassiana* isolates (Vandenberg *et al.*, unpublished).

Their results indicate that *P. xylostella* larvae vary in their susceptibility to infection by *B. bassiana* based on isolate, dose, age and temperature. They showed that an isolate originating from *P. xylostella* larvae was not significantly more efficacious than a commercial isolate. In field trials the commercial isolate provided >60% mortality when applied 2X week (Vandenberg and Shelton, unpublished) but these trials were not season-long. We are also investigating more of a niche market for using fungi. Previous studies have shown that *P. xylostella* are often brought into an area on transplants (Shelton *et al.*, 1996) and a key management strategy is to control them prior to transport. Preliminary studies have indicated that twice weekly foliar sprays of a commercial formulation of *B. bassiana* can provide adequate control of *P. xylostella* on seedlings and may provide another tool for resistance management.

Parasitoids

The importance of the larval parasitoids such as *Diadegma, Cotesia and Diadromus* spp. cannot be underestimated (Talekar and Shelton, 1993). More recently, work on *Trichogrammatid* egg parasitoids have shown promise. Pak (1992) has shown the value of testing *Trichogrammatid* spp. for their host specificity and suitability, searching ability, environmental requirements, reproductive capacity and rearing method. A laboratory evaluation of 27 *Trichogrammatid* spp. (Klemm *et al.*, 1992) has identified some promising species for use against diamondback moth. Additional work conducted by Vaquez and Shelton (unpublished) evaluated 6 commercially available species of *Trichogrammatid* parasitoids and found that *T. pretiosum, T. bactrae* and *T. minutum* consistently caused the highest levels of mortalities of *P. xylostella* (95–98%) in laboratory studies. We are currently testing these populations in the field in Honduras. Our laboratory is also working with colleagues in material sciences (Shelton, Craighead and Hoffmann, unpublished) to create mass-produced artificial eggs for *Trichogrammatid* rearing.

Other Insecticides

The literature on synthetic insecticides is abundant, as is the documentation of resistance to these insecticides (Talekar and Shelton, 1993). Some new classes of insecticides which are effective against *P. xylostella* are now being used. Avermectin products are currently registered in several areas, including southeast Asia, and have been shown to provide excellent control of *P. xylostella* and results to date have shown no cross-resistance to insecticides to which *P. xylostella* has become resistant, e.g. methomyl and permethrin (Lasota *et al.*, 1996). Other similar products, such as Spinosad, derived from microbes are under development for control of *P. xylostella*. The

latest generation of pyrethroid products has been highly effective at very low rates against *P. xylostella* which are still susceptible to the older pyrethroids. Products derived from novel chemistry, such as AC 303,630, have proven to be very effective against *P. xylostella* in field trials and may be used against *P. xylostella* which have developed resistance to most other products. Extracts of neem have also provided adequate control (Schmutterer, 1992) but the time to death may be of some concern for widespread adoption. These products may have their greatest potential for resource-poor farmers who could produce their own product from local trees.

Mating Disruption

Studies in Japan (Ohbayashi *et al.*, 1992; Ohno *et al.*, 1992), Taiwan (Chow 1992; Nemoto *et al.*, 1992), Canada (Chisholm *et al.*, 1984), and Florida (McLaughlin *et al.*, 1994) have shown that the orientation of the male diamondback moth to synthetic and natural pheromone sources can be disrupted by release of synthetic sex pheromones. The pheromone disruption technique may provide a reliable alternative to the sole use of insecticides. This tactic may also allow for the more effective integration of other management tactics (e.g., conservation of natural enemies and biological control) that are often incompatible with insecticide use. However, in our own trials in 1993 and 1994 our results were not as promising (Shelton and Schroeder, unpublished). In those trials we evaluated the continuous polyethylene 'rope' dispensers (Shin-Etsu Chemical Co., Tokyo, Japan) containing the sex pheromone of the diamondback moth--a 1:1 mixture of (Z)-11-hexadecenal and (Z)-11-hexadecen-1-ol acetate-- on reduction of larval infestations, trap catch and mating disruption. The relatively poor results we experienced and the high cost of this technology in its present form would not be appealing to growers. Advances in production of the pheromone and improved dispensers for it may overcome this limitation in the future.

Cultural Control and Plant Resistance

Rotation to non-crucifers and clean cultivation practices, although hardly novel, are the best management tactics which can be employed. Movement of *P. xylostella*-infested plants, especially transplants, from one area will often lead to uncontrollable problems (Shelton *et al.*, 1996), but this can be managed. More difficult to manage is the spatial separation of plantings and crop-free periods. In the broccoli growing regions of Mexico, an overall management program which included a crop-free period has been effective, but as enforcement of this crop-free period has been relaxed problems have resurfaced.

Trap cropping, a technique practised before the advent of modern insecticides, is now making a comeback in areas like India. In these situations mustard has been the preferred trap crop and becomes preferentially colonized by *P. xylostella* and the larvae remain in the crop and also become heavily parasitized.

Plant resistance has been reviewed previously (Talekar and Shelton, 1993). Two primary paths of work are under investigation. Transgenic plants expressing *B. thuringiensis* toxins has been noted above. The other path is the continued investigations into identifying the chemicals involved in surface waxes which elicit non-preference behaviour and how such behaviour lead to mortality of larvae because of their increased exposure to the environment and natural enemies. Work has also been initiated on engineering plants to modify their production of leaf surface waxes.

Conclusions

If there is anything we have learnt about *P. xylostella* it is that management with single-component strategies will fail. Emphasis needs to be placed on developing multiple strategies which are compatible and educating growers about the need for multiple approaches. These are the real challenges. Growers await the newest 'silver bullet' and they can be led to believe that such a tactic will get them by until something better comes along. Emphasis needs to be placed on developing resistance management strategies for the products that we presently have or ones that are being developed. Use of supplemental agents like microbials or narrow range synthetic insecticides may hold great possibility, but the backbone of any management program should remain sound cultural practices and use of natural enemies since these are the most durable practices.

References

Chisholm, M. D., E. W. Underhill, P. Palaniswamy, and V. J. Gerwing (1984). Orientation disruption of male diamondback moths (Lepidoptera: Plutellidae) to traps baited with synthetic chemicals or female moths in small field plots. *J. Econ. Entomol.* ***77:*** 157–160.

Chow, Y. S. (1992). Disruption effect of the synthetic sex pheromone and its analogs on diamondback moth, pp. 105–108. *In* N. S. Talekar [ed.], Management of diamondback moth and other crucifer pests: Proceedings of the Second International Workshop, Shanhua, Taiwan.

Humber, R. A. (1992). Collection of entomopathogenic fungal cultures: catalog of strains. U. S. Department of Agriculture, Agricultural Research Service, Ithaca, NY. Publication ARS-110.

Ibrahim, Y. B., and W. Low (1993). Potential of mass-production and field efficacy of isolates of the entomopathogenic fungi *Beauveria bassiana* and *Paecilomyces fumosoroseus* against *Plutella xylostella*. *Int. J. Pest Manag.* ***39:*** 288–292.

Klemm, U., M. F. Guo, L. F. Lai, and H. Schmutterer (1992). Selection of effective species or strains of *Trichogramma* egg parasitoids of diamondback moth, pp. 317–324. *In* N. S. Talekar [ed.], Management of diamondback moth and other crucifer pests: Proceedings of the Second International Workshop, Shanhua, Taiwan.

Lasota, J. A., A. M. Shelton, J. A. Bolognese, and R. A. Dybas (1996). Toxicity of avermectins to diamondback moth populations: implications for susceptibility monitoring. *J. Econ. Entomol.* ***89:*** 33–38.

Liu, Y., B. E. Tabashnik, and M. Pusztai-Carey (1996). Field-evolved resistance to *B. thuringiensis* toxin CryIC in diamondback moth. *J. Econ. Entomol.* ***89:*** 798–804.

Loke, W. H., G. S. Lim, A. R. Syed, A. M. Abdul Aziz, M. Y. Rani, M. Md. Jusoh, U. B. Cheah, and I. Fauziah (1992). IPM on the diamondback moth in Malaysia: development, implementation and impact, pp. 529–539. *In* N. S. Talekar [ed.], Management of diamondback moth and other crucifer pests: Proceedings of the Second International Workshop, Shanhua, Taiwan.

McLaughlin, J. R., E. R. Mitchell, and P. Kirsch (1994). Mating disruption of diamondback moth in cabbage: reduction of mating and suppression of larval populations. *J. Econ. Entomol.* ***87:*** 1198–1204.

Metz, T. D., R. T. Roush, J. D. Tang, A. M. Shelton, and E. D. Earle (1995). Transgenic broccoli expressing a *Bacillus thuringiensis* insecticidal crystal protein: a model system to test resistance management strategies. *Molecular Breeding* ***1:*** 309–317.

Nemoto, H., E. Yano, and K. Kiritani (1992). Pheromonal control of diamondback moth in the management of crucifer pests, pp. 91–97. *In* N. S. Talekar [ed.], Management of diamondback moth and other crucifer pests: Proceedings of the Second International Workshop, Shanhua, Taiwan.

Ohbayashi, N., K. Shimizu, and K. Nagata (1992). Control of diamondback moth using synthetic sex pheromones, pp. 99–104. *In* N. S. Talekar [ed.], Management of diamondback moth and other crucifer pests: Proceedings of the Second International Workshop, Shanhua, Taiwan.

Ohno, T., T. Asayama, and K. Ichikawa (1992). Evaluation of communication disruption method using synthetic sex pheromone to suppress diamondback moth infestations, pp. 109–114. *In* N. S. Talekar [ed.], Management of diamondback moth and other crucifer pests: Proceedings of the Second International Workshop, Shanhua, Taiwan.

Pak, G. A. (1992). Inundative release of *Trichogramma* for control of cruciferous Lepidoptera: preintroductory selection of an effective parasitoid, pp. 297–308. *In* N. S. Talekar [ed.], Management of diamondback moth and other crucifer pests: Proceedings of the Second International Workshop, Shanhua, Taiwan.

Pell, J. K., E. D. M. Macaulay, and N. Wilding (1993a). A pheromone trap for dispersal of the pathogen *Zoophthora radicans* Brefeld. (Zygomycetes: Entomophthorales) amongst populations of the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Yponomeutidae). *Biocontrol Sci. Technol.* ***3:*** 315–320.

Pell, J. K., N. Wilding, A. L. Player, and S. J. Clark. (1993b). Selection of an isolate of *Zoophthora radicans* (Zygomycetes: Entomophthorales) for biocontrol of the diamondback moth *Plutella xylostella* (Lepidoptera: Yponomeutidae). *J. Invertebr. Pathol.* ***61:*** 75–80.

Perez, C. J., A. M. Shelton, and R. C. Derksen (1995). Effects of field application technology and *B. thuringiensis* subspecies on management of *B. thuringiensis* subsp. *kurstaki*-resistant *P. xylostella. J. Econ. Entomol.* ***88:*** 1113–1119.

Perez, C. J., and A. M. Shelton (1996a). Resistance of *P. xylostella* to *B. thuringiensis* in Central America. *J. Econ. Entomol.* (in press as J96–061).

Perez, C. J., and A. M. Shelton (1996b). Field applications, leaf-dip, and diet incorporated diagnostic assays used against *B. thuringiensis*-susceptible and resistant diamondback moth. *J. Econ. Entomol.* (in press as J96–215).

Perez, C. J., J. D. Tang, and A. M. Shelton (1996a). Comparison of leaf-dip and diet bioassays for monitoring resistance in field populations of diamondback moth. *J. Econ. Entomol.* (in press as J96–101).

Perez, C. J., A. M. Shelton, and R. T. Roush (1996b). Managing diamondback moth resistance to foliar applications of *B. thuringiensis*: testing strategies in the field. *J. Econ. Entomol.* (submitted as J96–257).

Riethmacher, G. W., and J. Kranz (1994). Development of disease incidence of Entomophthoraceae in field populations of *Plutella xylostella* in the Philippines. *J. of Plant Diseases and Protection* ***101:*** 357–367.

Schmutterer, H. (1992). Control of diamondback moth by application of neem extracts, pp. 325–332. *In* N. S. Talekar [ed.], Management of diamondback moth and other crucifer pests: Proceedings of the Second International Workshop, Shanhua, Taiwan.

Shelton, A. M., J. L. Robertson, J. D. Tang, C. Perez, S. D. Eigenbrode, H. K. Preisler, W. T. Wilsey, and R. J. Cooley (1993). Resistance of diamondback moth to *Bacillus thuringiensis* subspecies in the field. *J. Econ. Entomol.* ***86:*** 697–705

Shelton, A. M., J. A. Wyman, N. L. Cushing, K. Apfelbeck, T. J. Dennehy, S. E. R. Mahr, and S. D. Eigenbrode (1993). Insecticide resistance of diamondback moth in North America. *J. Econ. Entomol.* ***86:*** 11–19

Syed, A. R. (1992). Insecticide resistance in the diamondback moth in Malaysia, pp. 437–442. *In* N. S. Talekar [ed.], Management of diamondback moth and other crucifer pests: Proceedings of the Second International Workshop, Shanhua, Taiwan.

Tabashnik, B. E., N. L. Cushing, N. Finson, and M. Johnson (1990). Field development of resistance to *Bacillus thuringiensis* in diamondback moth. *J. Econ. Entomol.* ***83:*** 1671–1676.

Talekar, N. S., and A. M. Shelton (1993). Biology, ecology and management of the diamondback moth. *Ann. Rev. Entomol* ***38:*** 275–302.

Tang, J. D., A. M. Shelton, W. J. Moar, and R. T. Roush (1995). Consequences of shared toxins in strains of *Bacillus thuringiensis* for resistance in diamondback moth. *Resistant Pest Management Newsletter, Vol. 7, No.* ***1:*** 5–7.

Tang, J. D., A. M. Shelton, J. Van Rie, S. De Roeck, W. J. Moar, R. T. Roush, and M. Peferoen (1996). Toxicity of *Bacillus thuringiensis*. spore and crystal protein to resistant diamondback moth. *Applied and Envir. Micro.* ***62:*** 564–569.

Vandenberg, J. D., and M. Ramos (1997). Screening of fungal isolates against larvae of the diamondback moth, 1995. *Arthropod Management.* Vol. 27 (in press).

Insecticide resistance management in diamondback moth: *quo vadis?*

Richard T. Roush

Department of Crop Protection, University of Adelaide, Glen Osmond, South Australia, 5064 Australia

Abstract

The diamondback moth has shown such a formidable capability to evolve resistance to insecticides that we must consider radically different approaches to management of both the pest and its resistance. These include: mandatory crucifer-free crop periods, area-wide insecticide rotation programs, avoidance of pesticide mixtures and Bt spray formulations containing multiple toxins, avoidance of persistent insecticide formulations, registration of insecticides that show low toxicity to natural enemies, development of novel control tactics such as pheromone disruption, and the use of transgenic plants with multiple toxins "pyramided" within the same variety. Although it is often assumed that resistance will develop quickly to transgenic plants, simulation models and experiments suggest that pyramided plants with effective toxin expression, if coupled with a small refuge of non-transgenic plants, could be the most effective resistance management tactic ever devised, and might control diamondback moth populations through the mid 21st century. Transgenic crops could also dramatically reduce worker and consumer exposure to pesticides. Given that both transgenic technology and 4-5 new insecticides are or could soon be available for the diamondback moth, it is both a critical and an ideal time for implementing resistance management: there is pesticide susceptibility to conserve!

Key words: transgenic crops, insecticide resistance, fipronil, pest management, *Plutella xylostella*

Introduction

For more than 40 years, the principal control tactic for the diamondback moth (*Plutella xylostella* L., Lepidoptera: Yponomeutidae) around the world has been the use of insecticides. However, this approach is neither desirable nor sustainable. The public has clearly indicated a preference for a reduction of pesticide use. Further, the diamondback moth itself is forcing an eventual reduction in pesticide use by overcoming insecticides. It has evolved resistance to all modern insecticides that have been used intensively for any length of time (Talekar and Shelton, 1993) and has become famous even among molecular biologists for its evolution of resistance to *Bacillus thuringiensis*, Bt (Tabashnik, 1994). Although there has been a recent flurry of new insecticide introductions, there is every expectation that these will also suffer from resistance. At least one study suggests that resistance will be rapid if an insecticide is used more than twice per cropping cycle (Tabashnik, 1986). Some of the new insecticides may be even more susceptible to resistance than some of the insecticides already in use. For example,. the new insecticide fipronil can suffer cross-resistance to dieldrin and endosulfan (Cole *et al*., 1995).

If continuing the same insecticide use practices isn't the answer, where do we go next? We have only one choice: we must adopt truly integrated practices for the management of the diamondback moth, of which insecticide resistance management is only a part. Shelton *et al*., (1997) summarised many of the possible non-insecticidal control tactics that might be used.

Tactics

Reduce insecticide use

The first and foremost rule of resistance management is to avoid insecticide use, especially to reduce the number of sprays made and the proportion of the pest population that is treated (Roush, 1989). This is of course easily said, but how do we achieve it?

Perhaps the single most important feature of any resistance management effort is a sampling scheme that eliminates unnecessary sprays, and targets sprays only to those areas where sprays are truly needed. In many regions, a key problem for diamondback moth control is that the pest densities must be nearly zero at harvest due to local "cosmetic" standards. In these areas, cost-effective sampling is probably infeasible near harvest. On the other hand, low densities can be tolerated early in the cropping cycle in every region, yet all too often sprays remain on a calendar basis. In the long term, insecticides can be effective just before harvest only if they are used sparingly or not at all during the early season.

As reported at this meeting, biological control by parasitic wasps is very effective at least in tropical highlands, and there is hope that new species or geographic strains, especially from South Africa, will prove helpful in other areas (Sivapragasam *et al*., 1997). Especially early in the cropping cycle, it is crucial to avoid mortality of natural enemies, through the use of selective insecticides or the selective application of insecticides. In this context, insecticides currently being brought to market (including fipronil, chlorfenapyr, diafenthiuron, fenoxycarb, emamectin, and spinosad) offer particular hope, as most appear to

be relatively "soft" on beneficials (Sivapragasam *et al*., 1997). If this proves the case, it will be especially important to slow resistance to these products to preserve them for IPM. Pathogens of diamondback moth may also have an important role (Shelton *et al*., 1997), especially granulosis viruses (Su, 1991).

However, at least so far as is currently known, none of the tactics listed above will consistently avoid frequent insecticide applications in all regions. More drastic steps are necessary, and for this we must look to unique weaknesses of the diamondback moth. The only weakness that seems readily manipulated is that diamondback moth can feed only on crucifers. In other species with such a limited host range, the local elimination of the host via crop rotation has proven to be a very effective pest and resistance management tactic, such as for another pest with a propensity for resistance, the Colorado potato beetle (eg., Roush *et al*., 1990). A mandatory crucifer-free period has been undertaken for diamondback moth control in regions of at least two countries, Mexico (Shelton *et al*., 1997) and Australia. After the introduction of a summer break in crucifer production in southern Queensland during 1990, frequencies of resistance to permethrin appeared to drop sharply (Heisswolf and Hargreaves, 1994; Heisswolf *et al*., 1996). At the Gatton Research Station in the Lockyer Valley, resistance appeared to stabilise, and at the Redlands Research Station in the Redlands Valley, at least the evolution of higher levels of resistance seems to have been delayed *(Figure 1)*.

As was the case in southern Queensland (Heisswolf and Hargreaves, 1994; Heisswolf *et al*., 1997), a mandatory break in crucifer production will never be easy to implement. Neither was crop rotation for the Colorado potato beetle, but growers who found a way to rotate their crops now have fewer insecticide resistance problems (Roush *et al*., 1990) and are able to rely on insecticides in those cases when they still need them. I suggest that an important part of the long term and sustainable future of crucifer production will be the establishment of grower cooperatives in neighbouring growing regions to produce crucifers (and complementary vegetable crops) in alternate times of the year to defeat the diamondback moth while stabilising crop markets. Regions which do this in the near future will remain highly profitable and retain access to relatively cheap and effective insecticides, whereas regions that cannot practise host-free periods will probably eventually suffer an economic decline. That insecticide resistance can often appear to be quite localised in diamondback moth (Tabashnik *et al*., 1987) implies that these regions would not have to be greatly distant from one another; even if the moths do move from one site to another, such dispersal should delay and thereby reduce pest reproduction and increase the chances of adult mortality, as in the Colorado potato beetle (see citations in Roush *et al*., 1990).

A more futuristic and unproven tactic for managing the diamondback moth is pheromone disruption (Shelton *et al.*, 1997). Pheromone disruption has the potential for maintaining diamondback moth densities at low levels once they are already low, and indeed probably starts to fail once moth densities exceed about 1 000 per hectare, assuming diamondback moth is similar to other moth species where pheromone disruption works (Roush, unpublished analysis). In spite of probably overly high field densities, there have been some modestly promising results for pheromone disruption on diamondback moth (Shelton *et al*., 1997). This tactic now seems even more promising in light of results from Eric Rumbo and Richard Vickers (Vickers, 1996) on a new blend that seems to collect about 10 times as many moths at traps as the 1:1 blend used previously.

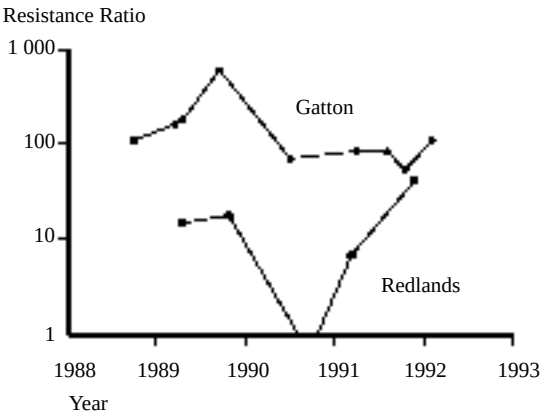


Figure 1. Apparent decline in resistance of diamondack moth at Gatton and Redlands Research Stations of southern Queensland following the break in summer production of crucifers commenced in the summer (January/February) of 1990. Data from Heisswolf and Hargreaves (1994)

Refinements of pesticide use

Where pesticides must be used, minor changes in use patterns can further delay resistance. First, avoid persistent pesticides or formulations, such as soil applications, which continue to select for resistance long after damaging densities of the pest have been eliminated (Roush, 1989). Also contrary to popular myth, there is no general advantage to applying high doses of insecticides, and indeed high doses may even inhibit biological control. The use of higher doses is especially inappropriate once resistance has been found in the field, because the strategy depends critically on a low initial gene frequency. The high dose strategy also assumes consistent and uniform high doses that kill greater than about 95% of the heterozygous (RS) insects, and no inhibition of susceptible migrants from untreated sites (refuges), assumptions that are usually not met by chemical sprays (Tabashnik and Croft, 1982; Roush, 1989; Roush 1994). Susceptible migrants are essential to "dilute" resistance among any individuals that survived the high dose.

In addition, rotating the use of pesticides over an entire area in a so-called "window strategy" tied to the calendar has proven to be a very effective resistance management tactic both in terms of adoption and efficacy (Roush, 1989; Forrester *et al*., 1993). The use

of different compounds at roughly the same time in neighbouring fields constitutes a mosaic of treatment patterns and should be avoided. Mosaics are simply the worst way to deploy a set of pesticides (Roush, 1989). The problem is that you have simultaneous selection with several pesticides, resulting in much lower pesticide durability (as illustrated in Roush, 1993). Not all pesticides will have similar efficacy. For example, Bt products may well have lower efficacy than the new synthetic insecticides, and may therefore be more appropriate to use early in the cropping cycle when the crop can withstand higher densities of larvae. However, this does not prevent us from adopting a window strategy for all products that are not so limited, placing them in the system at times that take advantage of their particular seasonal strengths, if any. Each new pesticide might be allotted a 1-3 month period depending on local conditions (eg., duration of the cropping season, which months had the fastest generations).

Perhaps most importantly, it is critical to avoid the use of spray mixtures. Contrary to another popular myth, it has long been clear that mixtures of insecticides do not necessarily delay resistance compared to the rotational or sequential use of the same insecticides. Experimental studies have failed to consistently find any advantage to mixtures (Tabashnik 1989, Immaraju *et al*., 1990), and theoretical models showed that mixtures will significantly delay resistance only when several conditions are met (Gould, 1986; Roush, 1989; Roush, 1997). To be most effective, mixtures require a lack of cross-resistance between the toxins, low initial frequencies of the resistance genes, refuges (as with the high dose strategy, such that resistant genotypes are rare and can be diluted), high mortality from each of the insecticides when used alone, a high spatial correlation of residues (not just equal decay rates), and it helps if at least one heterozygous genotype suffers high mortality and the two resistance loci are not closely linked (Roush, 1989).

Transgenic crops

Transgenic crops incorporating genes from Bt have already been commercialised in the USA (potatoes, maize, and cotton). Broccoli transformed with one of the "Cry1A" genes used in cotton and maize provides excellent control of Bt-susceptible diamondback moths, although not of Bt-resistant diamondback moths collected from areas of intensive Bt use (Metz *et al*., 1995). These plants were developed exclusively for research purposes, but plants transformed with other Bt genes would probably be effective even in areas of the world where resistance to Bt sprays is already widespread. Further, even plants with Cry1A genes would be useful in controlling other lepidopterous pests of crucifers that have not yet evolved resistance to Bt, and would thereby simplify pest management programs.

Insect resistant Bt-transgenic crops have often been considered to put Bt at risk due to their potentially persistent exposure of insect populations to Bt toxins. However, the high and relatively uniform expression of Bt toxins (and lack of impact on immigrating susceptible moths) means that transgenic plants can potentially overcome the shortcomings of the high dose and mixture strategies and delay resistance more effectively than sprays, especially where two or more transgenic resistance traits are pyramided into the same plant (Roush, 1994; Roush, 1997). The diamondback moth may be a case in point (Metz *et al*., 1995; Roush, 1994). Unfortunately, many people have considered transgenic plants to be no more than simply a new way to deliver an insecticide, with no potential for integration with other tactics. To the contrary, transgenic plants offer new potentials for integration.

Although it seems that people most often think of transgenic crops as the sole solution to pest control problems, transgenic crops should more usefully be thought of primarily as a means of slow pest population growth, to be used in concert with other pest control tactics. To delay resistance, there must be refuges of non-transgenic host plants to "dilute" resistance from any survivors of the transgenic crops, and these refuges should generally be as large as possible, such that 10-20% or more of the pest population develops on non-transgenic hosts (Roush, 1994; Roush, 1997). Although suitable refuges may often be achieved with non-crop hosts, in many cases crops will be the only suitable refuges, yet we don't want any crops to be damaged at levels that will cause commercial losses. One way to address this problem is to transform only those varieties that are most sensitive to damage or where the diamondback is most difficult to control (eg., brussel sprouts) and continue to use only non-transgenic varieties of crops where control is less critical. For example, relatively little control of diamondback moth may be needed at least in the early stages of crop development in cabbage and broccoli. If these were planted on a range of maturity dates, they could provide refuges for both Bt and insecticide susceptible diamondback moths unless and until they had to be sprayed near harvest.

Recall from earlier in this paper that pheromone disruption of mating is not effective when the pest population is too high (the males and females can find each other in spite of the pheromone), a problem that transgenic crops could address by restraining population growth. Pheromones in turn could allow larger and more effective refuges while limiting damage to the crop (Roush, 1997). Similarly, classically bred resistance traits (Shelton *et al*., 1997) within the transgenic crop could put additional stresses on Bt-resistant insects, slowing resistance and reducing the size of the refuge needed (Roush, 1994; Roush, 1997).

Model Programs?

It is important to combine all available tactics for an effective resistance management effort. An ideal

program that could be implemented today would combine a crucifer-free period, a reliance on parasites whenever possible, Bt use only when needed while the crop is early in its development, and as the crop approaches maturity, the careful use of synthetic insecticides only when needed and in a "window" rotational format.

In the future, the ideal program might add the use of transgenic varieties of only those crops that are most sensitive to damage, especially where the transgenic plants include multiple resistance traits, the use of pheromone disruption, and the use of insect pathogens where needed on the non-transgenic crops

Conclusions

This is an exciting time for resistance management of diamondback. Several new pesticidal and non-pesticidal controls are now available or may be developed. The danger is that people will become complacent with new pesticides and transgenic crops. If not fully integrated with other control tactics that are more difficult to implement, there is a real possibility that these new tools will be squandered and will fail to live up to their full potential to provide a sustainable system of pest management. Pesticide resistance management would perhaps more appropriately be called "susceptibility management". The new pesticides and transgenic technologies have revealed new types of susceptibility within populations; it is now up to us to manage them!

Acknowledgments

My understanding of crucifer pest management has benefited greatly from discussions with Tony Shelton, Bruce Tabashnik, Greg Baker, Mike Keller and N. S. Talekar.

References

Cole, L. M., Roush, R. T., and Casida, J. E. (1995). *Drosophila* GABA-gated chloride channel: Modified [^{3}H]EBOB binding site associated with Ala^{302}-> Ser or Gly mutants of *Rdl* subunit. *Life Sciences* ***56:*** 757-765.

Forrester, N. W., Cahill, M., Bird, L. J., and Layland, J. K. (1993). Management of pyrethroid and endosulfan resistance in *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Australia. *Bulletin of Entomological Research*, Suppl 1.

Gould, F. (1986). Simulation models for predicting durability of insect-resistant germplasm: a deterministic diploid, two locus model. *Environmental Entomology* ***15:*** 1-10.

Heisswolf, S., and Hargreaves, J. (1994). Managing diamondback moth resistance in south east Queensland. RIRDC National Workshop: Managing Resistance In Diamondback Moth. RIRDC, Melbourne, Australia, June 1994, pp. 5-10.

Heisswolf, S., Houlding, B. J., and Deuter, P. L. (1997). A decade of integrated pest management (IPM) in brassica vegetable crops- the role of farmer participation in its development in southern Queensland, Australia. *In:* Proceedings of Third International Workshop on The Management of Diamondback Moth and Other Crucifer Pests (29 October–1 November 1996).

Immaraju, J. A., Morse, J. G., and Hobza, R. F. (1990). Field evaluation of insecticide rotation and mixtures as strategies for citrus thrips (Thysanoptera: Thripidae) resistance management in California. *Journal of Economic Entomology* ***83:*** 306-14.

Metz, T. D., Roush, R. T., Tang, J. D., Shelton, A. M., and Earle, E. D. (1995). Transgenic broccoli expressing a *Bacillus thuringiensis* insecticidal crystal protein: implications for pest resistance management strategies. *Molecular Breeding* ***1:*** 309-317.

Roush, R.T. (1989). Designing resistance management programs: How can you choose? *Pesticide Science* ***26:*** 423-441.

Roush, R. T. (1993). Occurrence, genetics and management of insecticide resistance. *Parasitology Today* ***9:*** 174-179.

Roush, R. T. (1994). Managing pests and their resistance to *Bacillus thuringiensis*: Can transgenic crops be better than sprays? *Biocontrol Science and Technology* ***4:*** 501-516.

Roush, R. T. (1997). Managing resistance to transgenic crops. In Advances in Insect Control: The Role of Transgenic Plants (eds., N. Carozzi and M. Koziel) pp. 271-294. London: Taylor and Francis.

Roush, R.T., Hoy, C.W., Ferro, D. N., and Tingey, W.M. (1990). Insecticide resistance in the Colorado potato beetle (Coleoptera: Chrysomelidae): Influence of crop rotation and insecticide use. *Journal of Economic Entomology* ***83:*** 315-319.

Shelton, A. M., Perez, C. J., Tang, J. D., and Vandenberg, J. D. (1997). Prospects for novel approaches towards management of the diamondback moth. *In:* The Management of Diamondback moth and other Crucifer Pests. Proceedings of the Third International Workshop: (A .Sivapragasam *et al.*) pp. 17–20

Sivapragasam. A., W.H. Loke, A.K. Hussan and G.S. Lim (1997). The Management of Diamondback Moth and other Crucifer Pests. Proceedings of the Third International Workshop, 29 October –1st November, 1996, Kuala Lumpur, Malaysia.

Su, C-Y. (1991). Field trials of granulosis virus and *Bacillus thuringiensis* for control of *Plutella xylostella* and *Artogeia rapae*. *Chinese Journal of Entomology* ***11:*** 174-178.

Talekar, N. S., and Shelton, A. M. (1993). Biology, ecology and management of the diamondback moth. *Annual Review Entomology* ***38:*** 275-302.

Tabashnik, B. E. (1986). A model for managing resistance to fenvalerate in the diamondback moth (Lepidoptera: Plutellidae). *Journal of Economic Entomology* ***79:*** 1147-1151.

Tabashnik, B. E. (1989). Managing resistance with multiple pesticide tactics: theory, evidence, and recommendations. *Journal of Economic Entomology* 82, 1263-69.

Tabashnik, B. E. (1994). Evolution of resistance to *Bacillus thuringiensis. Annual Review Entomology* ***39:*** 47-79.

Tabashnik, B. E. and Croft, B. A. (1982). Managing pesticide resistance in crop-arthropod complexes: interactions between biological and operational factors. *Environmental Entomology* ***11:*** 1137-1144.

Tabashnik, B. E., Cushing, N. L., and Johnson, M. W. (1987). Diamondback moth (Lepidoptera: Plutellidae) resistance to insecticides in Hawaii: intra-island variation and cross-resistance. *Journal of Economic Entomology* ***80:*** 1091-1099.

Vickers, R. (1996). Personal communication. CSIRO Entomology, Canberra, Australia.

Bringing science to farmers: experiences in integrated diamondback moth management

Peter A. C. Ooi

FAO Intercountry Programme on IPC in Rice in South and Southeast Asia Jl. Taman Margasatwa No. 61, Jati Padang, Pasar Minggu Jakarta 12540, Indonesia

Abstract

There are five discernible phases in the evolution of an Integrated Pest Management (IPM) programme for the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) in Malaysia, namely: 1) Initial phase; 2) Intensification phase; 3) Crisis phase; 4) Disaster phase; and 5) IPM phase. This outline enables an understanding of the genesis of a farmer first approach to IPM. All five phases will be discussed and weaknesses of dominant approaches at each stage will be highlighted. As the evolution of a season long programme emerges, the similarity in development of IPM in other crops in the wet tropics will be discussed. Of particular interest to many national programmes are the similarities and differences between the two current approaches to implementing integrated diamondback moth management – one based on top down classroom lectures and messages and the other based on helping farmers understand ecology and getting farmers to make better pest management decisions. In support of a scientific approach, suggestions on bringing concepts of plant compensation (crop physiology), parasitism and predation to farmers are discussed. Concepts that are familiar to farmers, such as life cycle and microbial control are revisited. Experiences in Indonesia, Philippines and Vietnam have shown that a season long training of trainers is an important prerequisite to work with farmers. Farmers who learn about biological control of the diamondback moth are less likely to spray insecticides on cabbages in order to conserve the effective parasitoid *Diadegma semiclausum*.

Key words: *Plutella xylostella*, Integrated Pest Management, Implementation

Introduction

Traditionally, pest control is synonymous with taking actions to stop an infestation of a pest, and in this instance an insect. This is equivalent to a fire brigade function. With the advent of chemical insecticides in the early 1950s, this approach took on a simplistic remedial prescription. So prevalent is this approach that in many developing countries, insecticides are considered 'medicines' (Roling & de Fliert, 1994). There appeared to be little support to approach the problem ecologically, i.e., asking questions such as: why did the outbreak develop?, why is it that the particular insect occurs at a certain time and not others?, why is this insect common in one place but not in another geographical area? That the path taken was unsustainable is reflected in the present problems which resulted from the 'solution' initiated and adopted in the 1950s. This case study enables an analysis of paths chosen for diamondback moth management and reasons for choosing them as compared to other paths.

Looking back some 25 years ago, an introduction to pest control was associated with fitting the biological system to available technology. To this day, much remains the same even in the light of problems generated by the technology used. Farmers did not feature in the development of pathways leading to insect pest management. It is not surprising, therefore, that after more than 40 years since chemical insecticides dominated pest management, the issue has assumed a larger proportion when it became obvious that unless farmers are involved in the process of Integrated Pest Management (IPM), outbreaks and resurgence will continue. The lack of recognition of the role of farmers is described in the book by Chambers *et al.* (1989). That farmers could not be passive recipients of technology was shown by non-adoption in the 1950s and 1960s. Failure to adopt then was misinterpreted as farmers' inability to learn or ignorance. To solve this, extension education was developed to transfer technology to farmers. When non-adoption continued in the 1970s and early 1980s, it was attributed to farm-level constraints and hence the on-going effort to make farms more like research station administered fields. Similarly, in the 1980s, blame on failure to adopt shifted from farmers and farms to technology. Hence, an objective of this paper is to address this issue of adoption by farmers and suggest ways to improve farmers' productivity without stereotyping them as ignorant.

To address this question of farmers in pest management, the diamondback moth was selected for the case study based on the following considerations:

- it was the most important pest of cabbages in the region
- pest management efforts went through distinct phases
- effective management exists
- good historical account of the insect exists

- interest groups extended beyond farmers
- similar developments existed in all countries in the region

Development of IPM

Integrated Pest Management as a concept to solve pest problems came after it was realised that the panacea that was chemical control did not live up to its expectations and created more problems than it solved. Carson (1962) alluded us to this. Prior to the discovery of chemical insecticides, farmers relied on natural biological control and cultural practices including selecting varieties of plants that are more tolerant to pest damage. The science of pest management may be traced to the first successful intercontinental movement of beneficial insects in 1890. Here was a case where a problem was investigated, field observations and studies made and an understanding of the problem was realised. Investigators found that the cottony cushion scale, *Icerya purchasi* Maskell (Hemiptera: Margarodidae), was an introduced insect. It was not a pest in its native Australia and this soon led to the introduction of an effective predator and parasitoid into California. The cottony cushion scale population declined spectacularly after the introduction of these natural enemies in what became established as the first case of classical biological control (DeBach, 1974).

In contrast, let us discuss a hypothetical situation of the cottony cushion scale being discovered as a pest for the first time in the 1950s. Science would most likely take a backseat regarding management of this insect. What would most likely happen is that pest controllers would be engaged in evaluating various kinds of insecticides and sprayers to find the most dangerous insecticides to use on the insect. This technological approach, in contrast to the earlier scientific approach is the key difference in pathways adopted in finding solutions to pest problems.

The problems arising from use of chemical insecticides were realised as early as 1950s. By the early 1950s, resurgence of pest outbreaks and resistance to chemical insecticides were reported from all over the world. Initial attempts led to the first development of IPM as a combination of chemical and biological control (Stern *et al*., 1959). This did not go far enough to address the conflict of using two opposing approaches. Chemical insecticides by their very mode of action remove or inhibit the function of biological control and this poses a problem of integrating chemical and biological control. Recognising this, steps were then taken in the 1960s to formulate a more ecological approach (Smith & Reynolds, 1966). In the 1970s, the approach recognises the need to identify social and economic requirements in addition to ecological consideration (Bottrell, 1979). Despite all attempts at defining IPM, it remained very much at academic levels, particularly in the wet tropics. Part of this problem lies in the heavy reliance on the use of economic thresholds as a key element to decision making. Economic threshold levels were insensitive to both social and economic situations of farmers and it was realised by 1980s that the use of economic threshold levels continued to maintain unnecessary use of insecticides (Moore, 1996) due in large part to lack of understanding of underlying ecological principles.

This led to the development of a farmer first programme which helps farmers to understand the concept of ecosystem. Farmers will be able to identify causes of field problems, conduct scientific studies to further understand these causes and thereby becoming better pest managers who are capable of making good pest management decisions. We have come more than 40 years to arrive at this stage where we can consider farmers as partners in agricultural development. In summary the evolution of IPM has come a full cycle. It was in the hands of farmers but technocrats moved in by trying to develop IPM **for** farmers and when problems continue to crop up, it became apparent that development of IPM **by** farmers become more reasonable.

The diamondback moth – problems and solutions

The diamondback moth provides an interesting case study for a discussion on IPM development as the problem evolved at about the same time chemical insecticides were freely available. Five phases were observed which coincided with the way IPM developed in cotton (Smith & van den Bosch, 1967). This case study also provided an opportunity to compare development of integrated diamondback moth management with similar activities in cotton, rice and oil palm under tropical Asian conditions.

Stage 1: Initial Phase

The first phase pertains to the introduction of cabbages into tropical Asia. Both the cabbage and its accompanying traveller, the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), are exotic. In the initial phase, the insect established well in its new environment. At this stage (ca. 1920), cabbages were grown in home gardens in the highlands of Fraser's Hill and later in Cameron Highlands when it was opened in the 1930s. Any attempt at managing diamondback moth was based on crop rotation and use of botanical insecticides such as derris root extracts (Ooi & Sudderuddin, 1978). This phase could be compared to the cultivation of cotton or rice on a subsistence level in tropical Asia *(Table 1)*. The cabbage situation is closer to that of oil palm which like cabbage was introduced about two decades later. This phase is associated with generally low pest incidence.

Stage 2: Intensification Phase

It is possible that with the apparent lack of serious insect pests, intensified cabbage cultivation was encouraged. The discovery of tracts of land in Cameron Highlands in the 1930s suitable for agricultural activities led to increased cultivation of the vegetable. As this happened, an exotic pest such as *P. xylostella*

Table 1. Summary of evolution of pest management in cabbages as compared to cotton, rice and oil palm.

Phase	Cabbage	Cotton	Rice	Oil Palm
Initial	Crop introduced into cooler climates of tropics to provide for nutritional needs of locals	Cotton grown to supply local needs for millenia without serious problems	Rice cultivation existed for millenia in tropical Asia without serious pest problems.	Crop introduced from Africa under a commodity diversification programme.
Intensification	Increased area to supply vegetable needs of local and neighbouring countries. DBM introduced inadvertently. Control based on insecticides. No concern for sustainability. No ecological consideration.	More areas were cultivated to supply world and local consumption. Adopted dogma of increasing inputs to pursue higher production. No concern for sustainability. No ecological consideration	Countries increased areas under rice with irrigation and higher yield per area. Use of insecticides included in development packages. No concern for sustainability. No ecological consideration.	Plantation industry increased areas under oil palm. Some estates embarked on insecticide spray programmes to protect investment. No concern for sustainability. No ecological consideration.
Crisis	Complete dependence on chemical control led to evolution of insecticide resistance and resurgence of DBM. Insecticide usage amounted to half of total cost of production.	Reliance led to increasing use of insecticides, resulting in environmental degradation, evolution of insecticide resistance and resurgence of many pests. Cost of production increased.	Policies of subsidy led to increased use of insecticides, resulting in disruption of existing biological control. An unknown pest, BPH, resurged to become worse pest of rice.	Use of insecticide became standard practice in many estates. This led to environmental degradation resulting in outbreaks of bagworms and other native insects.
Disaster	Many crop failures. Frequent changes in types of insecticides. Farmers used mixtures. Consumers complained of residues.	Yields declined. Outbreaks of pests at epidemic levels. Declining area under cotton as farmers gave up in despair.	BPH outbreaks threatened food security in tropical Asia. Resistant rice varieties broke down with increasing use of insecticides.	This phase was avoided by the work of an ecological-minded entomologist
IPM	Ecological studies identified the need to import effective parasitoids. Farmers, under pressure from consumers switched to Bt, allowing established parasitoids to effectively reduced populations of DBM, in absence of chemical insecticides.	Ecological studies revealed the role of natural enemies. Sharp decline in use of insecticides. Yields and areas under cultivation increased.	Field ecological studies showed outbreaks of BPH caused by use of insecticides. Banning insecticides from rice fields combined with educating farmers about ecology helped make BPH a non-pest again.	Field ecological studies demonstrated that fields sprayed with persistent insecticides led to outbreaks and estates were discouraged from spraying. This stabilised the ecosystem and outbreaks declined.

Table 2. History of chemical insecticide usage for diamondback moth control in Malaysia - an update (adapted from Ooi, 1986 and Syed, 1992)

Insecticides	Year of introduction	Status
Nicotine & Derris	<1950	replaced by synthetic organic insecticides
DDT	1950	resistance suspected by 1956
Gamma HCH	1950	resistance suspected by 1956
Dieldrin	1950	not used on cabbages after 1956
Malathion	1956	resistance factor of 2096 by 1975
Dimethoate	1956	used mainly for leafminer control
Diazinon	1956	used in combination with other insecticides
Trichlorfon	1964	poor control by 1966
Isobenzan	1964	usage stopped by 1966
Dichlorvos	1966	unpopular with farmers
Methomyl	1967	unpopular by mid 1970s
Aminocarb	1969	resistance suspected by 1970
Quinalphos	1969	poor control by 1970
Leptophos	1970	poor control by 1971
Methamidophos	1970	used in combination with other insecticides
Cartap	1973	poor control by 1975
Bioresmethrin	1974	good initial control but unpopular
Fenvalerate	1975	usage reduced by 1979
Prothiofos	1976	used at high concentration by 1979
Permethrin	1978	resistance factor of >700 by 1980
Cypermethrin	1980	resistance developed due to cross-resistance
Diflubenzuron	1980	resistance noted by 1990
Chlorfluazuron	1986	resistance noted by 1990
Teflubenzuron	1987	resistance noted by 1990
Avermectin	1992	resistance noted by mid 1990s

multiplied without any impediment. In the late 1940s, efforts were made to control this pest with the new technology of that era, namely chemical insecticides. Farmers found that DDT, dieldrin and gamma-HCH were effective in killing pests and the euphoria of controlling nature probably prevented any attempt to view the problem ecologically (Ooi & Sudderuddin, 1978).

A similar development was observed in the case of cotton in Asia. As a result of political decisions to increase production in the 1970s, expansion of cotton cultivation was accompanied by increasing use of inputs such as chemical insecticides. In particular, use of chemical insecticides was apparently made in expectations that pest problems would increase with increased area under cultivation. This turned up to be a self fulfilling prophesy as increased use of insecticides resulted in pest outbreaks, starting in the late 1970s. There was no critical evaluation of the need to use chemical insecticides due to lack of concern for sustainability and ecology.

Similar development was observed in rice and again the lack of consideration of possible disruption due to chemical interventions was apparent. In particular, the switch to intensive cultivation was linked to programmes that included use of insecticides on a calendar basis with no evidence that such use was justified. In fact, double cropping in Malaysia actually saw a decline in damage by rice stemborers (Balasubramaniam & Ooi, 1977).

In the case of oil palm, with the discovery of an efficient oil extraction method, hectares under oil palm started to increase in the 1960s. Accompanying this is the perceived need to protect investments from potential pest damage. This led to estates subscribing to regular use of insecticides as part of estate practices with scant regard for sustainability and ecology.

Stage 3: Crisis Phase

By 1953, diamondback moth resistance to organochlorines was reported in Indonesia (Ankersmit, 1953). Farmers in Cameron Highlands also reported difficulties in controlling the diamondback moth by the mid-1950s (Henderson, 1957). However, despite cracks in the panacea of chemical control, advocates refused to discontinue the practice and instead sought for even more toxic organophosphates to replace the organochlorines *(Table 2)*. By 1976, the level of resistance to malathion was 2096 (Sudderuddin & Kok, 1978).

In the case of cotton, the crisis phase was reflected by insecticide resistance of a native insect, *Helicoverpa armigera* (Hubner) (Lepidoptera: Pyralidae) to existing insecticides in both India and China by the 1980s. In Vietnam, use of insecticides exceeded 20 times per season in the 1970s (Tho, pers. comm., 1996). Despite evidence of looming problems with use of insecticides, there was little attempt to remove reliance on chemical insecticides.

Outbreaks of the brown planthopper, *Nilaparvata lugens* (Stal) (Hemiptera: Delphacidae) started to develop in the early 1970s. Curiously, these happened initially in countries where there were policies of pesticide subsidies included in national rice programmes. These were initially attributed to rice intensification programmes and hence fulfilled the

prophesy for need to use insecticides. Later, susceptible varieties, asynchronous planting and weather were blamed for the outbreaks which became more intensive following increasing use of insecticides. A crisis had definitely developed.

The link to insecticide disruption was also not apparent initially in outbreaks of oil palm bagworms. This led to further outbreaks when farmers were trapped in the insecticide treadmill of using more insecticides to control resurgent outbreaks.

Stage 4: Disaster Phase

Despite regular changes of insecticides, farmers found the diamondback moth increasingly difficult to manage. Resistance developed quickly in the case of synthetic pyrethroids (Teh *et al.*, 1982). Yet another example is the development of resistance to a new class of insect growth regulators (IGRs). Outbreaks of the diamondback moth were recorded in 1988 and it was suspected that resistance to IGRs had developed (Syed, 1992). A feeling of desperation led to farmers experimenting with cocktails of insecticides which further exacerbated the problem (Ooi & Sudderuddin, 1978). Cost of use of insecticides in the Cameron Highlands had risen in excess of 40% of cost of production (Lim, 1972) and soon, more and more farmers had given up growing cabbages. In the Philippines, desperation drove farmers to use cyanide in an effort to control a pest which proved itself to survive despite all efforts to exterminate it. These were clear indications of a disaster phase. Consumers were concerned about the possibility of eating excessive insecticides and demands for cabbages dropped, thereby resulting in further shrinkage of cultivated areas under cabbages.

Cotton farmers in Shandong, China had to resort to 30 sprays of cocktails containing up to five different insecticides (Ooi, 1994). So strong was the dosage that leaf scorch due to insecticides was common. In the late 1980s and early 1990s, outbreaks of *H. armigera* were common in both India and China. Farmers had access to cheap and very toxic insecticides and out of desperation resorted to cocktails of these insecticides to control an insect which until recently led an innocuous existence. The outbreaks resulted in severe crop losses following 20 to 30 applications of insecticides. The losses were so severe that some cotton farmers in India committed suicide and in China many farmers gave up growing cotton altogether. Despite all this, researchers and pest managers in major cotton growing countries continued to promote use of economic thresholds and forecast outbreaks of *H. armigera* based on regular monitoring of only the herbivore. Both technology promoted continued use of insecticides. When the level of control was not achieved, researchers searched for other technologies which were mainly non-ecological alternatives including use of synthetic pheromones to determine time of application of insecticides and prescribed use of insecticides, under an insecticide resistance management programme.

A similar experience was reported in rice in some southeast Asian nations. Continued breeding of resistant varieties to BPH provided short term reprieve and in cases where farmers resorted to regular use of insecticides, these varieties broke down quickly. Hence, the real cause of outbreaks were not identified and in many countries authorities continued to support an insecticide-centred programme. The BPH was considered the worse pest of rice but most researchers then, failed to understand why and in some cases actually promoted programmes that maintained the use of chemical insecticides thereby sustaining continuous outbreaks of the insect. This included use of economic threshold levels, early warning systems which determine time for application of insecticides and insecticide subsidy. Food security in some countries where rice is the staple food was threatened and these governments were hard pressed to solve the problem. The brown planthopper issue has then reached a crisis phase.

The oil palm case study provided an example of how a timely ecological intervention prevented the industry from drifting into a disaster phase. From a crisis phase, the situation moved quickly into an IPM phase.

Stage 5: IPM Phase

With clear signs of disaster in diamondback moth management in Cameron Highlands, ecological studies were initiated in 1975 which led to the realisation that *P. xylostella* lacked effective natural enemies (Ooi, 1979; Lim, 1982). This led to the introduction of effective parasitoids from New Zealand and Australia between 1976–78 (Ooi & Lim, 1989). Farmers were not involved then in the analysis of the problem and its possible resolution. This meant that despite the establishment of the parasitoids in Cameron Highlands, there appeared no benefits as the parasitoid populations were regularly killed by excessive use of chemical insecticides (Ooi, 1985). This persisted until consumer pressure brought to bear on the authorities to prevent excessive residues of insecticides in cabbages. Impetus to reduce insecticide residues was provided by an embarrassing situation of shipments of cabbages being destroyed upon arrival into a neighbouring country due to excessive residues. The Department of Agriculture of Malaysia reacted quickly to reinstall consumer confidence by imposing farm-gate residue testing. This move forced cabbage farmers to abandon rampant and incessant use of cocktails of insecticides and switch to products containing *Bacillus thuringiensis* (Bt). There was a surge in use of Bt in Cameron Highlands and farmers soon realised that the population of diamondback moth declined over the months that Bt use increased. There was scepticism that the decline was temporary and was attributed to weather, government actions etc. For three years the population stayed low and in some farms visited, farmers explained that they were surprised that when they stop using insecticides completely, diamondback moth also disappeared (Ooi, 1992). Little was done to explain

how *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae) kept populations of diamondback moth low. Some farmers were lectured on what the parasitoid looked like and it was accepted by many researchers and extension workers that farmers need not understand how the parasitoid worked as long as it controlled the insect pest!

Cotton IPM in Asia is still very much in Phase IV although attempts at initiating Phase V have already started (Ooi, 1994; Ooi & Kenmore, 1995). Initial results of this attempt have been encouraging.

Rice IPM provides a model for a successful implementation of a farmer-first approach to keeping pest outbreaks down. In the initial years, rice IPM follows the existing paradigm of components, i.e. IPM is the sum total of different approaches, however incompatible these may be. In the 1980s, use of economic threshold levels was still considered fashionable and featured in almost all literature concerning IPM. It was realised then, that this did not work out as farmers did not understand the concept of injury level and had problems counting bugs. With the exception of Malaysia (Ooi, 1982), surveillance systems in Asian countries focused on populations of herbivores and decisions were made based on numbers of pest species. This encouraged farmers to use insecticides in anticipation of pest species reaching economic threshold levels. To exacerbate the problem, farmers failed to recognise the beneficial species and often considered them as pests (Winarto, 1995). All these served to argue against a top down approach. Hence, when the President of the Republic of Indonesia wanted a sustainable approach to rice production without reliance on chemical insecticides, a farmer-first programme evolved to take over from a dysfunctional, knowledge-intensive, top down programme (Wardhani, 1992). Farmers do not need to memorise economic threshold levels nor do they need to remember what are the insecticides banned by the President for use on rice. In addition, they do not have to sit through boring lectures but get into the field to discover science and learn about the ecosystem and factors that kept the BPH populations low over the millennia. Studies were made to learn about the effective natural enemies through experiments. Human resource development was the main driving force of the programme.

As with rice, ecological studies were conducted to confirm that spraying insecticides caused outbreaks on oil palm (Wood, 1964; 1973). With such evidence, it was easy to sell a cost-effective management programme to the plantation industry and this has become the mainstay of the industrys' approach to pest management. Malaysia was among the first countries in the world where scientific evidence were made available to growers to change from an existing paradigm of 'insurance' application of insecticides. Science prevailed over technology and the industry grew to help the country in its economic growth.

Learning from the diamondback moth experience

From the rice and oil palm experiences, we learn to appreciate the important role of existing natural biological control. However, the diamondback moth experience added a new dimension of an exotic pest. Here, we realise the need to introduce effective natural enemies, originally from the same place as the diamondback moth. However, the problem is not solved by just releasing the parasitoids into the new environment. Farmers are part of the pest management scenario and have to be involved. In the Philippines, despite rearing and releasing large numbers of *D. semiclausum* into the Cordilleras, there was little impact as long as farmers continued spraying regularly (Poelking, 1992). This was similar to the situation in the Cameron Highlands, Malaysia (Ooi, 1992) and Indonesia (Sastrosiswojo & Sastrodihardjo, 1986) *(Table 3)*. The problem of continued usage of insecticides as an impediment to effective establishment of *D. semiclausum* was also discussed by Talekar (1992)

It is suggested that classical biological control programmes, for diamondback moth or other insect pests, should involve farmers right from the beginning. Farmers who understand the ecological basis of the programme will be most likely to co-operate in the establishment of the parasitoid and its eventual conservation following establishment. This can be achieved through a non-formal education approach which emphasises learning by carrying out experiments and understanding the ecosystem through a season-long programme. A cadre of skilled trainers are needed to facilitate this activity. These trainers are educated in a similar way.

When the parasitoid finally showed its ability in the Cameron Highlands in the late 1980s to keep diamondback moth populations low *(Figure 1)*, following farmers' preference for Bt, efforts were made to extend this information to farmers. Many short sessions were conducted to tell farmers about biological control. It is uncertain how well farmers learn under classroom conditions but the return to using very toxic compounds suggest that this mode of teaching did not have a good impact. Hence, despite the presence of an effective biological control agent, farmers did not understand how it works.

It is suggested that top down messages may not be a suitable way of educating farmers about the ecosystem. Lessons from rice IPM have suggested that farmers who understand biological control are less likely to use chemical insecticides regularly. Learning by conducting comparative studies to understand the impact of biological control agents will go a long way to sustain IPM practices which minimises use of chemical insecticides and hence helps in the management of insecticide resistance. Farmers learn best when they are in their own fields and working with their crops.

It has been recommended that mixtures of insecticides should be avoided as these often accelerates resistance (Sun, 1992). However, it has

Table 3. Status of classical biological control of diamondback moth in southeast Asian countries (compiled from Sastrosiswojo & Sastrodihardjo, 1986; Ooi, 1992; Talekar, 1992; Poelking, 1992)

Country	Year of introduction	Status
Indonesia	1950	Attempts to bring in *D. semiclausum* were initiated since 1920 but finally succeeded in the 1950s. The parasitoid was sent to other parts of the country.
Malaysia	1975	Established in Cameron Highlands but impact realised only after 1989 following slowdown in use of chemical insecticides
Philippines	1989	Established in the Cordilleras but impact realised only after 1994 following efforts to educate farmers using a non-formal education approach to understanding ecology
Thailand	1989	Failed to establish in the field, probably due to excessive use of insecticides and few suitable highlands for release and establishment
Vietnam	1996	Recently introduced to both Hanoi and Dalat an attempts are being made to involve farmers in the biological control programme through IPM field schools

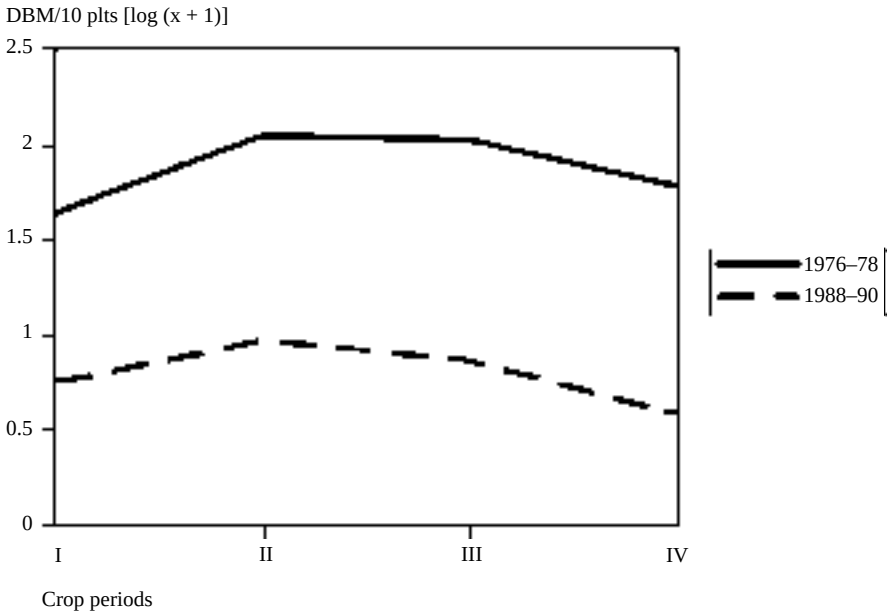


Figure 1. Population trends of diamondback moth (DBM) in Cameron Highlands from 1976–78 (prior to release of parasitoid), compared with that for 1988–90 following widespread establishment of Diadegma semiclausum, demonstrating successful biological control (Ooi, 1992). Each period represents ca 3.5 weeks equivalent to a life cycle of the diamondback moth in the Cameron Highlands.

been shown that farmers resorted to this practice out of desperation (Ooi & Sudderuddin, 1978). This observation suggests that farmers often conduct experiments to solve pressing problems in their fields. However, farmers are able to experiment on what they know and complete reliance on chemical control meant that farmers could only experiment on ways to improve this approach. It is not surprising that farmers found that mixing insecticides provided some measures of control. As farmers did not understand the concept of insecticide resistance, it appears logical that they react to poor control by increasing dosages and frequency of sprays.

It is suggested that farmers be provided the opportunity to learn science and understand concepts related to crop cultivation. Again, experiences from educating farmers in rice IPM demonstrated that farmers learn better about concepts of ecosystem, predation *(Table 4)* and crop physiology *(Table 5)* through direct experimentation in farmers' fields. A myopic reliance on chemical insecticides with neglect

for field ecology has led to widespread development of insecticide resistance.

A season-long training programme for farmers should not be viewed as the main objective of an extension programme. Rather, the training provides an introduction to an empowerment process and farmers should be encouraged to continue the process of discovery after the training.

The aim of a season-long training is to learn the process of conducting studies in order to discover and not to be passive recipients of instructions. However, the learning process continues long after the formal training. To sustain the scientific basis of IPM requires follow up activities. Bringing science to farmers should be the goal of IPM training. An inquiring mind, supported by skilled trainers who act as resource persons, will make farmers better farm managers.

Acknowledgements

The author is grateful to the organisers for the kind invitation to share his experiences with the participants. It is hoped that lessons learnt from tropical Asia will help develop a better way of approaching pest problems. Ideas for the development of this ecological approach evolved through much discussion with my friends: Peter E. Kenmore, Brian J. Wood, Ikram Mohyuddin, Lim Guan Soon, Jeff Waage, Khoo Soo Ghee, Khoo Khay Chong to name a few. I wish to thank the CTA of the FAO Intercountry Programme for permission to participate in the workshop.

References

Ankersmit, G. W. (1953) D.D.T. resistance in *Plutella maculipennis* (Curt.) (Lepidoptera) in Java. *Bulletin of Entomological Research* ***44:*** 421–425.

Bottrell, D. G. (1979) *Integrated Pest Management* Council of Environmental Quality, Washington, D. C. 120 pp.

Balasubramaniam, A. & Ooi, P. A. C. (1977) Status of rice stem-borers following double-cropping in Krian, Malaysia. *Malaysian Agricultural Journal* ***51:*** 54–61.

Carson, R. (1962) *Silent Spring*. Penguin Books, Harmondsworth. 317 pp.

Debach, P. (1974) *Biological control by natural enemies.* Cambridge University Press, London. 323 pp.

Chambers, R., Pacey, A. & Thrupp, L. A. (eds.) (1989) Farmer innovation and agricultural research. Intermediate Technology Publ. 218 pp.

Henderson, M. (1957) Insecticidal control of the diamondback moth (*Plutella maculipennis* Curt.) on cabbages at Cameron Highlands. *Malayan Agricultural Journal* ***40:*** 275–279.

Ho, T. H. (1965) The life-history and control of the diamond-back moth in Malaysia. Bulletin of the Division of Agriculture, Ministry of Agriculture and Co-operatives, Malaysia No. 118.

Lim, G. S. (1982) The biology and effects of parasites on the diamond-back moth, *Plutella xylostella* (L.). Ph. D. thesis, University of London, United Kingdom.

Lim, K. P. (1972) Vegetables production in Cameron Highlands - an economic study. *The Malayan Agriculturist* ***10:*** 14–23.

Moore, M. (1996) Redefining Integrated Pest Management – Farmer empowerment and pesticide use reduction in the context of sustainable agriculture. In: *Growing Food Security – Challenging the link between pesticides and access to food*. pp. 79–86. Pesticides Trust/PAN.

Ooi, P. A. C. (1979) Incidence of *Plutella xylostella* (L.) (Lepidoptera:Yponomeutidae) and its parasite, *Apanteles plutellae* Kurdj. (Hymenoptera:Braconidae) in Cameron Highlands, Malaysia. *Malaysian Applied Biology* ***8:*** 131–143

Ooi, P. A. C. (1982) Attempts at forecasting rice planthopper populations in Malaysia. *Entomophaga* 27 (Special Issue): 89–98

Ooi, P. A. C. (1985) Diamondback moth in Malaysia. In "*Diamondback moth Management*" (eds. Talekar & Griggs) AVRDC, Taichung, Taiwan pp. 25–34

Ooi, P. A. C. (1992) Role of parasitoids in managing diamondback moth in the Cameron Highlands, Malaysia. In "*Diamondback moth and other crucifer pests*" Proceedings of the Second International Workshop (ed. N. S. Talekar) AVRDC, Taichung, Taiwan pp. 255–262.

Ooi, P. A. C. (1994) Technical progress report No. 1. Integrated Pest Management in Cotton (RETA 5514) (May – October, 1993) for Asian Development Bank and CAB International. 11 pp + 3 appendices + 21 tables (mimeo).

Ooi, P. A. C. & Lim, G. S. (1989) Introduction of exotic parasitoids to control the diamondback moth in Malaysia. *Journal of Plant Protection in the Tropics* ***6:*** 103–111

Ooi, P. A. C. & Kenmore, P. E. (1995) Lessons for Vegetable IPM from rice and cotton. Presented and Compiled as the Report of the ASEAN Seminar-Workshop on Integrated Pest Management Training on vegetables

Table 4. Studies from insect zoo to learn about common predators found in cabbages in Hanoi and number of diamondback moth larvae (small) eaten per day.

Predator	No. eaten per day
Lycosa sp.	3–6
Ladybeetle adult	3–6
Ladybeetle larva	4–5
Paederus fuscipes	1–4
Earwig	2–6
Syrphid larva	3–6

Table 5. Some results of defoliation studies conducted with cabbages in Hanoi to help farmers better understand plant compensation in relation to insect feeding

Treatment	Removal at				
	7 DAT	14 DAT	21 DAT	28 DAT	35 DAT
25%	8.3%*	2.5%	6.3%	1.4%	16.0%
50%	9.0%*	10.2%	6.3%	9.1%	28.6%

* – % of shortfall in yield as compared to untreated

Production, 13–17 November, 1995, Lembang, Bandung, Indonesia, Volume 1 (7 pp.)

Ooi, P. A. C. & Sudderuddin, K. I. (1978) Control of diamond-back moth in Cameron Highlands, Malaysia. In *Proceedings of Plant Protection Conference* (eds. Amin, L. L. et al.) pp. 214–227. Rubber Research Institute of Malaysia, Kuala Lumpur.

Poelking, A. (1992) Diamondback moth in the Philippines and its control with *Diadegma semiclausum*. In "*Diamondback moth and other crucifer pests*" Proceedings of the Second International Workshop (ed. N. S. Talekar) AVRDC, Taichung, Taiwan pp. 271–278.

Roling, N. & van de Fliert, E. (1994) Transforming extension for sustainable agriculture: The case of Integrated Pest Management in rice in Indonesia. *Agriculture and Human Values* ***11:*** 96–108.

Sastrosiswojo, S. & Sastrodihardjo, S. (1986) Status of biological control of diamondback moth by introduction of parasitoid *Diadegma eucerophaga* in Indonesia. In "*Diamondback moth Management*" (eds. Talekar & Griggs) AVRDC, Taichung, Taiwan pp. 185–194.

Smith, R. F. & Reynolds, H. T. (1966) Principles, definitions and scope of Integrated Pest Control. In: Proceedings of the FAO Symposium on Integrated Pest Control, FAO, Rome. pp. 11–17.

Stern, V. M., Smith, R. F., van den Bosch, R. & Hagen, K. S. (1959) The integration of chemical and biological control of the spotted alfalfa aphid. The Integrated Control concept. *Hilgardia* ***29:*** 81–101.

Sudderuddin, K. I. & Kok, P. F. (1978) Insecticide resistance in *Plutella xylostella* collected from the Cameron Highlands of Malaysia. *FAO Plant Protection Bulletin* ***26:*** 53–57.

Sun, C. N. (1992) Insecticide resistance in diamondback moth. In "*Diamondback moth and other crucifer pests*" Proceedings of the Second International Workshop (ed. N. S. Talekar) AVRDC, Taichung, Taiwan pp. 419–426.

Syed, A. R. (1992) Insecticide resistance in diamondback moth in Malaysia. In "*Diamondback moth and other crucifer pests*" Proceedings of the Second International Workshop (ed. N. S. Talekar) AVRDC, Taichung, Taiwan pp. 437–442.

Talekar, N. S. (1992) Integrated Pest Management of diamondback moth: a collaborative approach in Southeast Asia. In *Integrated Pest Management in the Asia-Pacific Region* (eds. Ooi, *et al.*) pp. 37–49. CAB International, Kuala Lumpur.

Teh, P. C., Sudderuddin, K. I. & Ng, S. M. (1982) Toxicological studies of permethrin on the cruciferous pest, *Plutella xylostella* L. In "*Proceedings of International Conference on Plant Protection in Tropics*" (eds. Heong, K. L. *et al.*) Malaysian Plant Protection Society, Kuala Lumpur pp. 399–406.

Wardhani, M. A. (1992) Developments in IPM: the Indonesian case. In *Integrated Pest Management in the Asia-Pacific Region* (eds. Ooi, *et al.*) pp. 27–35. CAB International, Kuala Lumpur.

Winarto, Y. T. (1995) State intervention and farmer creativity: Integrated Pest Management among rice farmers in Subang, West Java. *Agriculture and Human Values* ***12:*** 47–57.

Wood, B. J. (1964) Severe outbreaks of caterpillars on oil palm estates in Malaya induced by the use of residual contact insecticides. In *Proceedings of the XII International Congress of Entomology*. p. 575.

Wood, B. J. (1973) Integrated Control: Critical assessment of case histories in developing economies. In *Insects: studies in population management*. Geier, P. W. *et al.* (eds.) Ecological Society of Australia (memoirs 1), Canberra. pp. 196–220.

Seasonality of major cabbage pests and incidence of their natural enemies in Central Kenya

G. I. Oduor[1], B. Lohr[2] and A. A. Seif[2]

[1]National Agricultural Research Centre Muguga, KARI, P.O. 30148, Nairobi, Kenya
[2]GTZ IPM Horticulture Project, P.O. Box 41607, Nairobi, Kenya

Abstract

The population dynamics of major brassica pests and occurrence of their natural enemies were monitored weekly for a period of 9 months on cabbage (var. Copenhagen Market) at a research station plot (no pesticides applied) and a farmer's field (weekly application of Permethrin 10 EC or Dimethoate 40 EC) in Central Kenya. Pest infestations occurred within 14 days after the seedlings were transplanted and persisted thereafter. Diamondback moth (DBM) (*Plutella xylostella*) and cabbage aphid (*Brevicoryne brassicae*) were the major pests at the two sites. At the station, the average population of DBM encountered were mainly larval-pupal parasitoids including *Diadegma* sp. and *Oomyzus sokolowskii* whose combined parasitism rarely exceeded 20% at any one time. The mean combined parasitism for the three growing seasons was about 7% with *Diadegma* sp. being more dominant (60.5%). Mean infestation by cabbage aphid for three seasons was about 41%. The mean rate of parasitism of the aphid by *Diaretiella rapae* was about 1.2%. In the farmer's field, the average population of DBM larvae and pupae per plant were 1.12 and 1.2, respectively. Natural enemies encountered included *Diadegma* sp., *O. sokolowskii*, bacteria and entomopathogens whose mean combined parasitism for the two seasons was about 7%. *B. brassicae* was also the most common aphid with mean infestation and parasitism of about 26 and 0.8%, respectively, for the two seasons. Other commonly observed pests included leafminers (*Liriomyza brassica*), *Thrips* sp., loopers (*Plusia* sp.) and foliar diseases. No parasitoids of DBM eggs were observed at the two sites.

Key words: *Plutella xylostella, Brevicoryne brassicae,* natural enemies, cabbage, Kenya.

Introduction

Cabbage (*Brassica oleracea* var. *capitata*) is a popular vegetable crop in Kenya. It is grown throughout the year by small-scale farmers in central and western districts of the country. The commonly grown varieties include Copenhagen Market, Giant Diant Drumhead, Savoy and Sugarloaf. The total hectarage of the crop is about 25,826 ha with a production of 266,467 tonnes valued at Kenya £126 038 891 (MALDM, 1994).

One major constraint to the production of cabbage in Kenya are pests, particularly, the diamondback moth (DBM) *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) (Madumadu *et al*., 1991). Like in some other parts of the world, the control of pests of cabbage in Kenya relies mainly on the use of chemical pesticides. However, repeated use of these pesticides elsewhere has led to the selection of pesticide resistance in DBM to the available pesticide arsenal (Bell and Fenemore, 1990; Hama, 1990; Zoebelein, 1990; Talekar, 1992). This necessitates a rethink in the control strategies currently employed in Kenya, and therefore, control measures which reduce dependence on pesticides need to be developed.

This paper presents preliminary results of a study on seasonality of major pests of cabbage and incidence of their natural enemies in Central Kenya with emphasis on DBM. This information is important to complement a biological control program for DBM.

Materials and Methods

The study was conducted between November 1995 and June 1996 at the National Agricultural Research Centre, Muguga, Kenya Agricultural Research Institute, (1° 13' South, 36° 38, 2096 m asl) (on-station) and at farmers' fields (on farm) 30 km away.

In the on-station trial, cabbage variety Copenhagen Market was planted every two months alternately in one of the three 50 x 10 m plots at a spacing of 50 x 65 cm. Diammonium phosphate (DAP) was used at planting and the plants were watered during the dry season. The plots were weeded two to three times in the growing season and no pesticides were applied to the plants. The trial was conducted for three consecutive seasons.

Weekly sampling at the on-station and on-farm plots was started two weeks after transplanting. Each of 20 randomly selected plants was examined for pests and diseases. The total numbers and stages of development of the lepidopteran pests observed on each plant were recorded. Emphasis was placed on the DBM. The populations of aphids (*Brevicoryne brassica, Lipaphis erysimi* or *Myzus persicae*) were estimated based on the following population score: 1 = no aphids, 2 = a few, 3= several colonies, 4 = half of a leaf covered with aphids and 5 = whole leaf covered with powdery/sooty mould. The presence of other pests was also recorded.

Some larvae and pupae of the lepidopteran pests were collected and individually placed in plastic containers (4 cm diameter x 3 cm high) with tight fitting lids. The former were reared on cabbage leaves in the laboratory. Colonies of aphids were cut from infested leaves, placed in 9 cm diameter plastic Petri dishes and fed with fresh cabbage leaves every other

day. These pests were observed daily for parasitoids and/or pathogens. Emerged parasitoids were labelled, preserved in clear gelatin capsules in plastic tubes and placed on the laboratory bench. However, diseased pests in gelatin capsules were stored in a refrigerator at 4 °C.

To study field parasitism of DBM eggs, 30 moths (males and females) were collected from the field using aspirators and released into nylon mesh cages (50 x 50 x 50 cm) containing four potted cabbage seedlings. The moths were allowed to oviposit on the seedlings for a period of 24 h. The seedlings were thereafter carefully examined and the areas bearing the eggs were marked using a felt pen. The seedlings with the eggs were then transferred to the cabbage plots (on-station/ on-farm) and exposed to parasitoids for two days. Leaves having the eggs were then removed from the field, the cut ends of their petioles placed in moist cotton wool, and maintained in covered plastic Petri dishes in the laboratory. All eggs were counted and monitored daily for emergence of parasitoids or larvae. Emerged larvae were reared to adult stage to check further for parasitoids. The experiment was done during the 2nd and 3rd growing season at the on-station plots and for two seasons at farmers' fields.

Meteorological data was taken from Muguga Meteorological Station sited at the National Agricultural Research Centre, Muguga. This information was considered not appropriate for the on-farm plots which were about 30 km away from the meteorological station, and therefore, was not used in this study.

Since the information presented is preliminary and the study is still on-going, the data obtained were not analyzed statistically.

Results

On-Station: The major pests were DBM and aphids. DBM was found to infest cabbage plants two weeks after transplanting. However, its population was low during the three growing seasons not exceeding two larvae/pupae at any one season *(Table 1)*. Percent parasitism was likewise low throughout the seasons being less than 20% at any one time. The parasitoids observed were *Diadegma* sp. and *Oomyzus sokolowskii.*

The relationship between DBM population, parasitoids and climatic data during the three growing seasons are illustrated in *Figures 1–3*. It is apparent that DBM population is inversely related to amount of rainfall as reflected in *Figures 2* and *Figure 3*. However, DBM population quickly picked up when the rains subsided.

Relationship between temperature and DBM populations could not be clearly discerned as the weekly average temperature differences were small (12.2–15.8 °C). The trend in parasitism reconciled with DBM larval populations *(Figures 1–3).*

Cabbage plants were also infested with aphids but the population score never exceeded 3 (several colonies). The population of *B. brassicae* was on the average higher than of *L. erysimi*. No *M. persicae* were observed during the three seasons *(Table 2)*. Percent infestation by *B. brassicae* and *L. erysimi* was on average about 41 and 29%, respectively. Both the species were parasitized by *Diaretiella rapae* and the mean percent parasitism for the three seasons was about 1.2%. However, the level of parasitism increased from one season to the next *(Table 2)*. In addition, a few hoverfly (Syrphidae) larvae were observed feeding on the aphids.

Table 1. Incidence of diamondback moth on cabbage (var. Copenhagen Market) and its parasitism at on-station and on-farm plots in Central Kenya (1995)

	Mean number				
	On-station (no-sprays)			On-farm (weekly sprays)[a]	
	Growing seasons				
	1	2	3	1	2
Larvae/plant	0.7	0.2	1.1	1.8	0.4
Pupae/plant	0.3	0.6	0.2	2.1	0.3
Level of parasitism (%)[b]	7.5	8.2	3.7	6.5	7.5

[a]Weekly application of Permethrin 40 EC (1st season) and Dimethoate 40 EC (2nd season)
[b]Pooled parasitism by *Diadegma* sp. and *Oomyzus sokolowskii*; figures for on-farm also included bacteria and fungi

Other insect pests observed on cabbage included leafminers (*Liriomyza brassica*), thrips (*Thrips* sp.), cutworms (*Agrotis* sp.) and loopers (*Plusia* sp.). Also taken into account was incidence of cabbage diseases of which the most common were ring spot (*Mycosphaerella brassicicola*), downy mildew (*Peronospora parasitica*) and black rot (*Xanthomonas campestris* pv. *campestris*) *(Table 3)*.

Quest for DBM egg parasitoids proved negative; none of 91 eggs exposed to parasitization in the field for 48 h yielded parasitoids.

On-farm: Populations of DBM in the on-farm plots were higher than in on-station despite of the weekly insecticidal sprays with Permethrin 10 EC or Dimethoate 40 EC *(Table 1)*. The mean number of larvae and pupae in two growing seasons was 1.8 and 0.4 and 2.0 and 0.3 per plant, respectively. Changes in DBM population and rates of parasitism in two seasons are depicted in *Figures 4* and *Figure 5*. The number of larvae reached up to about 6 per plant early in the season but gradually declined to about 1 per plant as the crop matured. However, the number of pupae increased with time.

Similar to the on-station plots, the main parasitoids recovered included *Diadegma* sp. and *O. sokolowskii*. The former was predominant. Parasitism rates are given in *Table 1*. In the second season insect pathogens were encountered [bacteria (2.8%) and fungi (2.6%)].

Aphids were fewer than in the on-station plots *(Table 2)*. *B. brassicae* was the major species, and *M.*

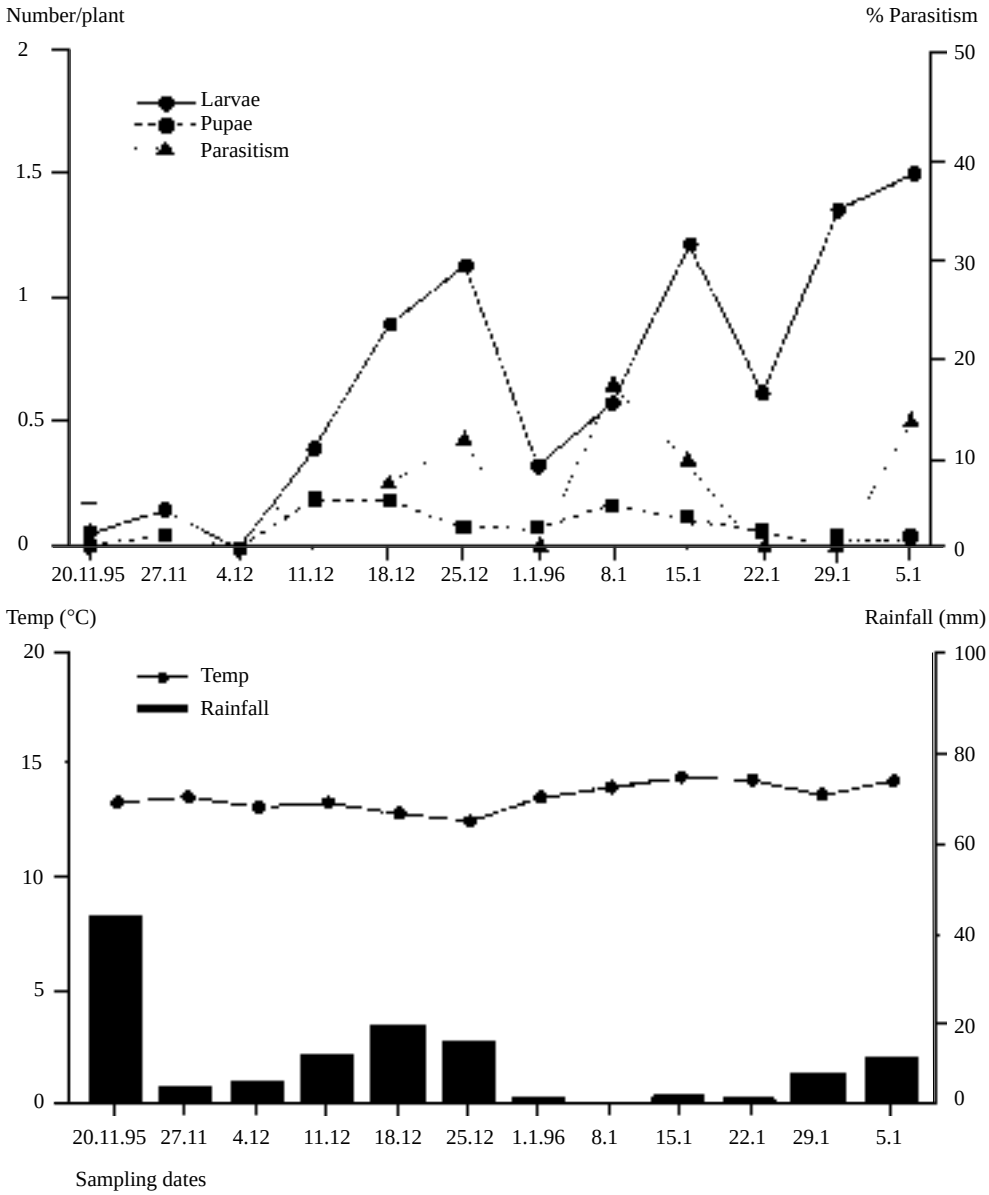


Figure 1. Population dynamics and percent parasitism of diamondback moth (top) and prevailing weather conditions (bottom) at the no-spray on-station field of cabbage during the first growing season in Central Kenya

persicae was not found on the crop. *D. rapae* was observed attacking aphids in the first season during which weekly sprays of Permethrin 10 EC were applied. In the second season Dimethoate 40 EC was used following which a few aphids were observed and no parasitoids were recovered.

In addition to DBM and aphids, a same complement of insect pests and cabbage diseases was recorded albeit at varying levels *(Table 3).*

A total of 102 DBM eggs were exposed for 48 h to parasitization in the field. No parasitoid was recovered as all the eggs hatched and developed into adults.

Discussion

On the basis of this study it is apparent that diamondback moth (*P. xylostella*) and aphids (*B. brassicae and L. erysimi*) are the major pests of cabbages in Central Kenya. The two were observed consistently for three seasons at on-farm and on-station fields with and without insecticidal application, respectively. However, DBM populations during the three seasons at both sites were generally low not exceeding 6 larvae/plant and there was hardly any difference in larval numbers between sprayed and no-spray fields. Furthermore, it was observed that rainfall suppressed DBM population. Probable explanation of the above could be a low population pressure during the material time of the study due to prevailing unfavourable weather conditions particularly rainfall and temperature despite the possible development of pesticide resistance in DBM to Permethrin 10 EC and Dimethoate 40 EC at the weekly sprayed fields. Similar results were reported in Taiwan (Anonymous, 1991) and in other Far-East countries (Chen and Su, 1986; Harcourt, 1986; Ong and Soon, 1989; Talekar, 1992).

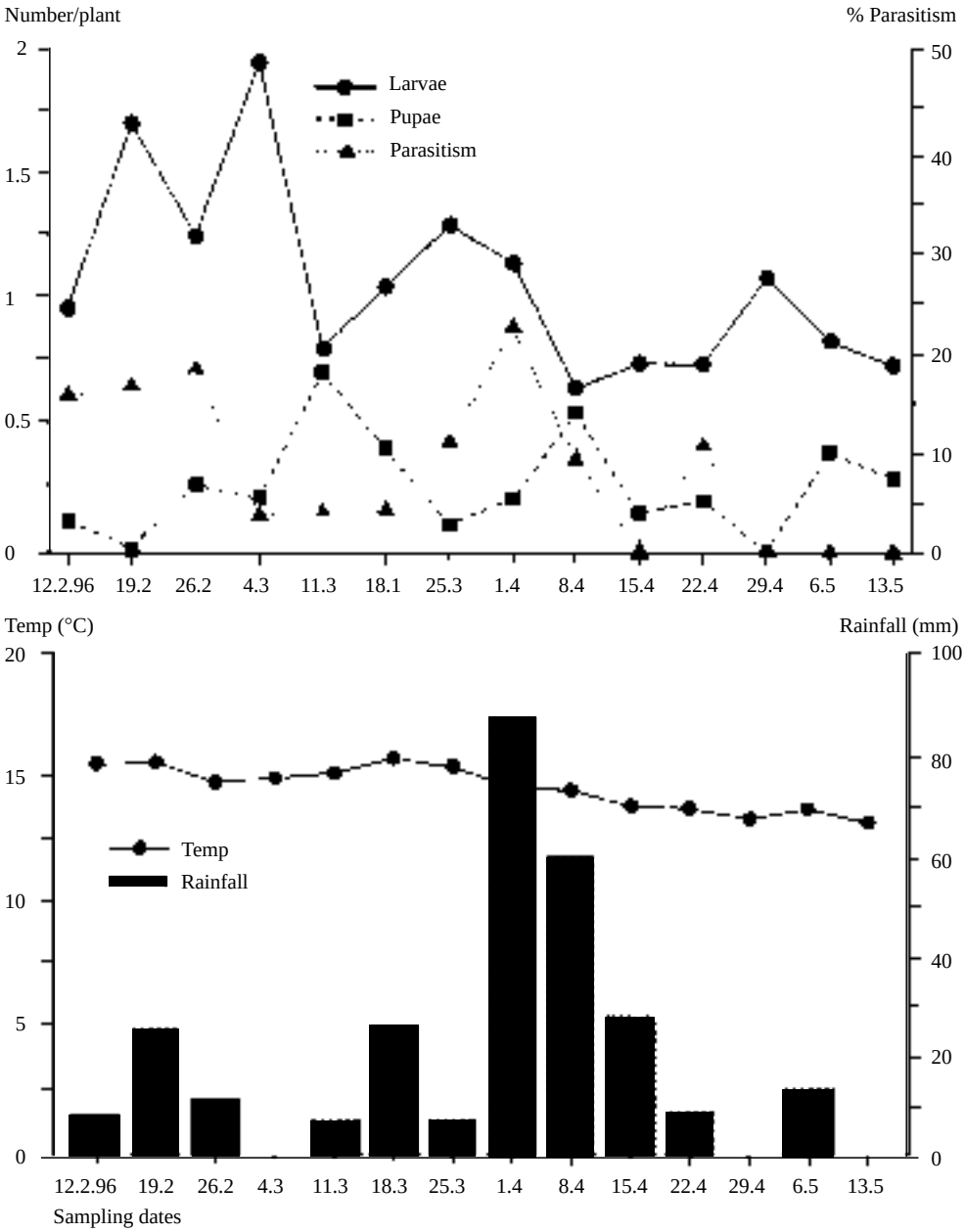


Figure 2. Population dynamics and percent parasitism of diamondback moth (top) and prevailing weather conditions (bottom) at the no-spray on-station field of cabbage during the second growing season in Central Kenya

In this study it was found that parasitism rates on DBM in all fields, irrespective of crop protection practices employed, were low. This was particularly evident in on-station fields where no pesticides were applied and could be attributed to the topography of the sites which were up-hill and windy. Such an environment was apparently unfavourable to the build-up of the populations of natural enemies of DBM. The major local parasitoids recovered from all the fields included *Diadegma* sp. and *Oomyzus sokolowskii* of which the former was predominant. Isolated cases of bacterial and fungal pathogens were encountered. Because of low numbers of natural enemies their impact on DBM population could only be minimal. In addition, no egg parasitoids were found in any of the sites. However, the study will be repeated at different areas within the Central Kenya and, will also be undertaken in other agroecological zones for proper inventory taking and impact assessment of the indigenous natural enemies of DBM.

Aphid populations consisting of mainly *B. brassicae* and *L. erysimi* were higher in no-spray than in pesticide sprayed fields. In the latter instance permethrin and dimethoate effectively controlled both species but at the same time dimethoate drastically reduced the rate of parasitism by *D. rapae*. Surprisingly, *M. persicae* was not observed on cabbage plots despite its high populations on adjacent Irish potato crop. A tangible explanation to such an incidence could be host preference: that is the aphid preferred potatoes to cabbages.

It is hoped that the results of this study once duly completed will aid in formulating appropriate management strategies for the major pests of cabbage

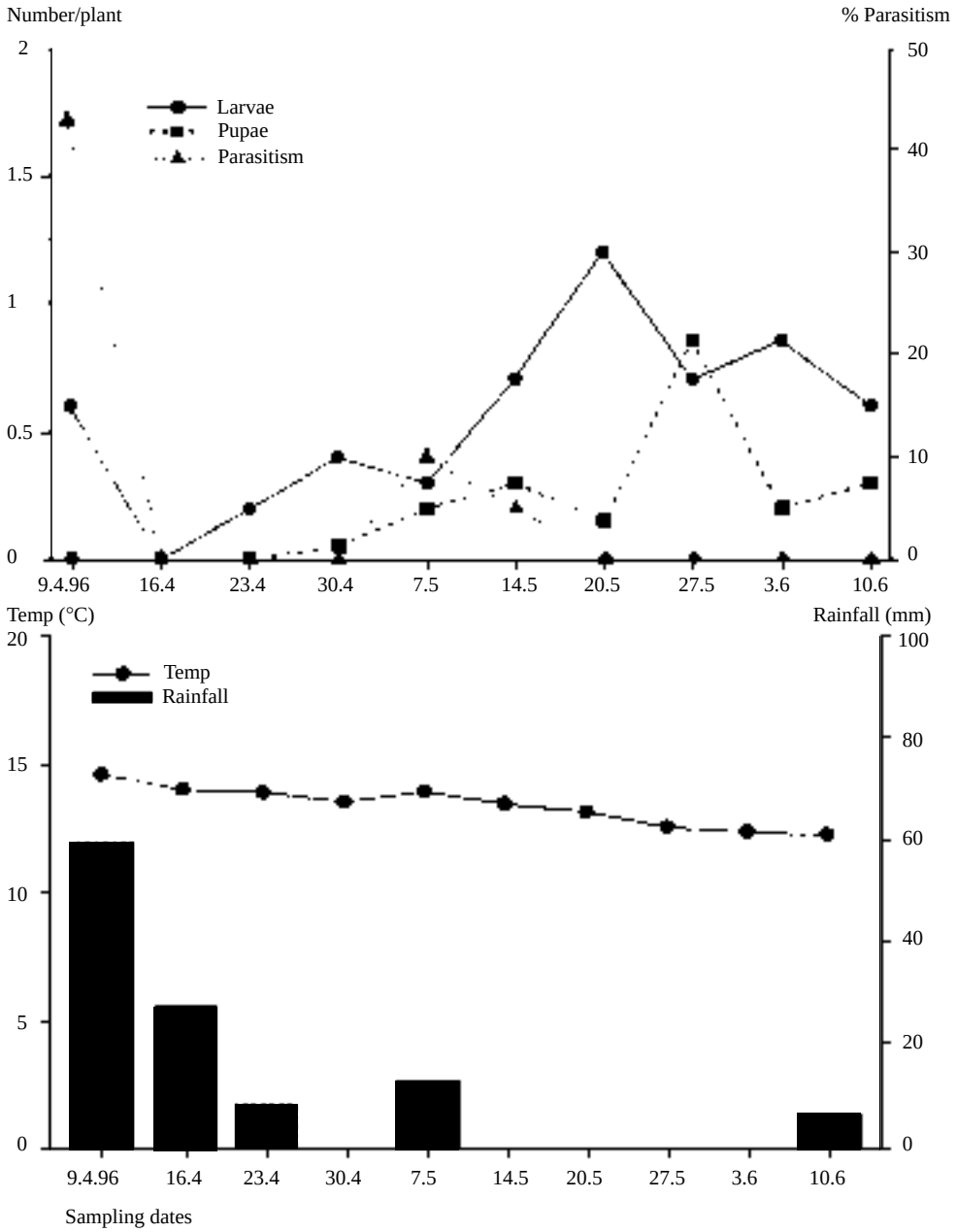


Figure 3. Population dynamics and percent parasitism of diamondback moth (top) and prevailing weather conditions (bottom) at the no-spray on-station field of cabbage during the third growing season in Central Kenya

in Kenya. Information on the inventory and impact of indigenous natural enemies will give guidance to a rational decision on whether to import exotic parasitoids (*Diadegma semiclausum* and/or *Cotesia plutellae*) for classical biocontrol of DBM in Kenya.

References

Anonymous (1991). Effects of insecticides on the parasitism of diamondback moth by two larval parasitoids. *Progress Report of Asian Vegetable Research and Development Centre* pp. 26–29

Bell, P. D. and P. G. Fenemore, (1990). Insecticide resistance in diamondback moth in New Zealand. *Proceedings of the Forty Third New Zealand Weed and Pest Control Conference* Palmerston 1990 pp. 31–34

Chen, C. N. and W. Y. Su, (1986). Ecology and control thresholds of the diamondback moth on crucifers in Taiwan. *Proceedings of the First International Workshop on Diamondback Moth Management,* Tainan 1986 pp. 415–421

Hama, H. (1990). Insecticide resistance of diamondback moth, *Plutella xylostella* in Japan. *Japan Agricultural Research Quarterly* ***24:*** 22–30

Harcourt, D. G. (1986). Population dynamics of the diamondback moth in Southern Ontario. *Proceedings of the First International Workshop on Diamondback Moth Management.* Tainan 1986 pp. 3–15

Madumadu, G. G., E. O. Sikinyi, and D. K. Mwamba, (1991). Vegetables for domestic and export markets. *Proceedings of Review Workshop on National Horticultural Research Programme* Thika Kenya 1991 pp. 41–63

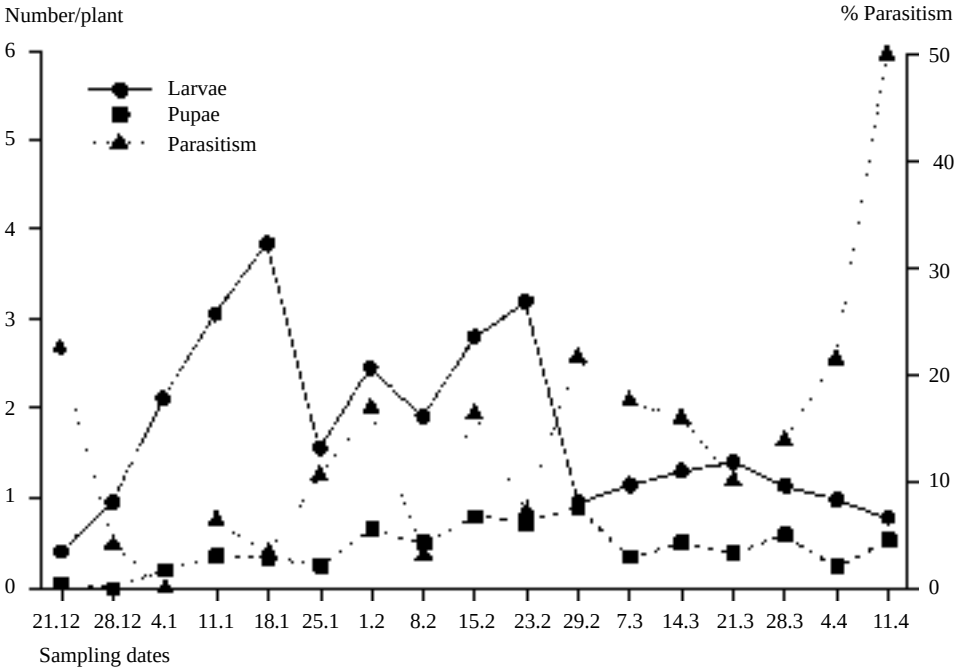


Figure 4. Population dynamics and percent parasitism of diamondback moth at the sprayed on-farm field during the first growing season

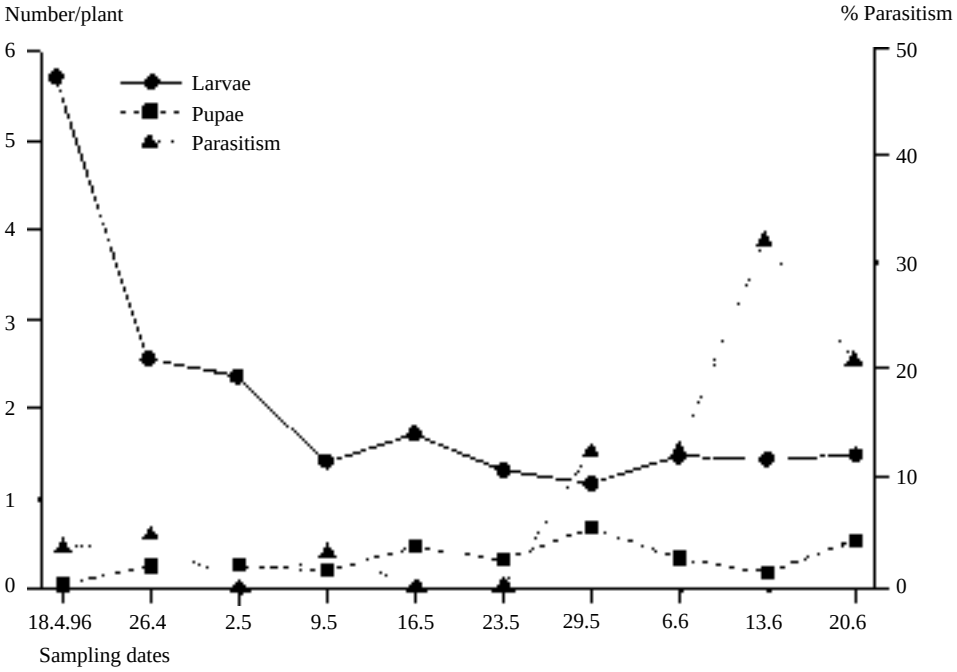


Figure 5. Population dynamics and percent parasitism of diamondback moth at the sprayed on-farm field during the second growing season

Table 2. Incidence, population score and parasitism of three aphid species infesting cabbage (var. Copenhagen Market) at on-station and on-farm plots in Central Kenya (1995)

Growing season		*Brevicoryne brassicae*	*Lipaphis erysimi*	*Myzus persicae*
On-station:				
1.	Plants infested (%)	26.7	41.2	0.0
	Mean population score[a]	1.4	1.5	1.0
	Mean parasitism (%)	0.6	1.5	0.0
2.	Plants infested (%)	54.3	16.2	0.0
	Mean population score	1.6	1.2	1.0
	Mean parasitism (%)	1.0	0.5	0.0
3.	Plants infested (%)	42.0	29.5	0.0
	Mean population score	1.5	1.3	1.0
	Mean parasitism (%)	1.9	1.6	0.0
On-farm:				
1.	Plants infested (%)	48.2	6.2	0.0
	Mean population score	1.6	1.1	1.0
	Mean parasitism (%)	1.7	0.0	0.0
2.	Plants infested (%)	3.5	11.0	0.0
	Mean population score	1.0	1.1	1.0
	Mean parasitism (%)	0.0	0.0	0.0

[a]Population score for aphids: 1= no aphids, 2= a few, 3= several colonies, 4= half of leaf covered with aphids and 5= severe attack (leaf covered with powdery/sooty mould)

Table 3. Incidence of other pests on cabbage (var. Copenhagen Market) at on-station and on-farm plots in Central Kenya (1995)

Pests	Mean incidence (%)	
	On-station (no sprays)	on-farm (weekly sprays)[a]
Arthropod pests:		
Liriomyza brassicae	61.3	33.4
Thrips sp.	25.9	45.4
Agrostis sp.	6.2	0.0
Plusia sp.	0.7	4.5
Plant diseases:		
Mycosphaerella brassicicola	46.6	59.0
Peronospora parasitica	22.1	13.6
Xanthomonas campestris pv. *campestris*	6.1	6.9

[a]Refer to *Table 1*

MALDM (1994). Crop production statistics. Ministry of Agriculture, Livestock Development and Marketing. *Annual Report* 1994 Kenya

Ong, K. H. and S. C. Soon, (1989). Seasonal abundance and distribution pattern among host plants of diamondback moth, *Plutella xylostella* (L.). *Singapore Journal of Primary Industries* ***17:*** 29–40

Talekar, N. S. (1992). *Diamondback Moth and Other Crucifer Pests: Proceedings of the Second International Workshop*, AVRDC Tainan 1990: 603 pp

Zoebelein, G. (1990). Twenty three year surveillance of development of insecticide resistance in diamondback moth (*Plutella xylostella* L., *Lepidoptera, Plutellidae*) from Thailand. *Madedelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent* ***55:*** 313–322

Diamondback moth in the north of Vietnam and proposal of control programme

Le Van Trinh
Entomology Division, National Institute of Plant Protection (NIPP), Hanoi, Vietnam

Abstract

The diamondback moth (DBM) *(Plutella xylostella* L.*)* is a major pest of cruciferous vegetables in Vietnam. If not controlled, DBM can reduce yields of cabbage and cauliflower by 30–40%, even by 100% in late season. At present, farmers control DBM by intensive pesticide use. Insecticides are applied in every 5–7 days, giving up to 12–15 sprays in a crop season of 70–80 days.

The study on DBM in the North of Vietnam showed that there were 16 population peaks during the year and durations between two peaks lasted from 15 to 31 days. Within the cabbage or cauliflower growing season, there were 10 peaks and population density in the field increased from August to April (of following year). Rainfall appeared to play dominant role in determining the population. *Apanteles plutellae* has a suppressing effect of 3.5–13.6% on the population of this insect. With a crop season of 70–80 days, there were 2 peaks of DBM populations in early (growing in July) and late crop season (growing in February) and 3 peaks in main crop season (growing in October). Measures applied to control DBM proved to be effective and are considered to be important in an IPM programme which consists of: sanitation after harvesting, intercropping cabbage/cauliflower with tomato and onion, pesticide use at peaks of 1st and 2 nd instar larvae at the time of 20–25, 45–50, 70–75 days after growing involving use of *Bacillus thuringiensis* (Bt) in early and main season, Bt and diafenthiuron in late season.

Key words: DBM, Vietnam, seasonal incidence, parasitoids, IPM

Introduction

The diamondback moth (DBM), *Plutella xylostella*, (L), (Lepidoptera; Yponomeutidae) is still considered to be the most serious pest of cruciferous crops in Vietnam. The yield losses due to it for cabbage, cauliflower, and other crucifers are usually from 30 to 40%, even up to 100% in the late growing season.

To protect crops from damage, farmers apply insecticides in every 5–7 days, giving up to 12–15 sprays in a crop season within 70–80 days.

One of reasons for the intensive insecticide use, and low control effectiveness is the lack of knowledge on its biology and ecology. The key elements of an effective control programme of the pest have not been properly established.

Material and Methods

Life history study

Investigations on the biology of DBM were carried out in the laboratory of Entomology Division, NIPP, Vietnam. Temperature and relative humidity (r.h.) during the period were variable depending on the environmental conditions. The adults started copulating almost immediately after emerging from the pupae that were collected from unsprayed fields. After laying eggs in insect-breeding cages on leaves of cabbage, the eggs with the leaf were left and put in Petri dishes for emerging. The larvae that emerged were bred on fresh cabbage leaf which was changed everyday until pupating. Each pair was individually studied and the number of eggs and durations of each developmental stage were counted and determined for each pair.

Field observation

Sampling techniques for estimating the DBM population and its parasites was undertaken every 5 days throughout the year. Thirty plants per plot were observed and numbers of the insect in each developmental stage and parasites plus other natural enemies were counted.

Results and Discussion

Life Cycle

The life cycle from egg to egg, on the average, took 21 days at mean temperature and relative humidity of 21.7°C and 83.5%, respectively. The egg period lasted 3.9 days, the larval durations from first instar to fourth were: 3.0; 2.6; 2.3; 2.4 days, respectively, pupae: 5.6 days and the pre-oviposition period was: 1.0 day. However, the life cycle took 27.1 days at 18.9 °C and 75.9% r.h. and even reached 33.1 days at 17.2 °C and 84.5% r.h. These results proved that the duration of the developmental stages and the life cycle of DBM clearly fluctuated depending on environmental temperature, but seemed not to be affected by relative humidity between 75.9% to 84.5% (see *Table 1*).

Fecundity

When the larvae were fed with cabbage leaf, each female laid an average 145.0 ± 18.9 eggs. The maximum oviposition by a female was 245 and minimum 62 eggs. The eggs reached 92.1%

Table 1. Duration of developmental stages and life cycle of diamondback moth (days)

Developmental Stage	1st Experiment (21.7°C; 83.5% r.h.)	2nd Experiment (18.9°C; 75.9% r.h.)	3rd Experiment (17.2°C; 84.5% r.h.)
Egg	3.9 + 0.3	4.7 + 0.4	4.8 + 0.4
1st instar larvae	3.0 + 0.2	3.4 + 0.1	4.9 + 0.2
2nd instar larvae	2.6 + 0.1	3.0 + 0.1	3.2 + 0.2
3rd instar larvae	2.3 + 0.1	3.0 + 0.1	3.6 + 0.2
4th instar larvae	2.4 + 0.1	4.1 + 0.2	7.0 + 0.2
Pupae	5.6 + 0.1	8.0 + 0.1	8.0 + 0.2
Preoviposition	1.0	1.0	1.0
Life cycle	20.8 + 0.2	27.1 + 0.2	33.1 + 0.3
Adult + Male	8.3 + 1.3	8.2 + 0.3	10.0 + 0.2
+ Female	7.0 + 1.09	7.0 + 0.2	8.6 + 0.2

Table 2. Fecundity of diamondback moth reared on cabbage leaves of different stages

Growth stage of plant	Eggs per female			Fertile eggs (%)
	Average	Maximum	Minimum	
Leaf development	145.0+18.9	245	62	92.1
Preheading	160.6+14.8	239	85	94.9

Note: During the experiment, average temperature was 24.3 °C, relative humidity was 83.0%

Table 3. Correlation (r) values between the duration between peaks and the density at peaks and temperature, relative humidity and rainfall.

Climatic components	Duration between peaks (r)	Density at peaks (r)
Mean temperature	–0.88	–0.20
Relative humidity	–0.02	–0.09
Rainfall	–0.28	–0.45

Note: - Number of peaks : 35
- The insect density: 0.2 – 24.9 individuals per plant

Table 4. Percentage of diamondback moth parasitised by *A.plutellae* in cabbage grown in winter

Growing season	Growing period	% DBM affected by *A.plutellae* at peaks		
		Peak 1	Peak 2	Peak 3
	Farmer's field			
Early	August – October	1.6	2.1	–
Medium	November – January	2.4	2.8	7.9
Late	February – April	6.5	10.2	–
	Unsprayed field			
Early	August – October	3.5	8.0	–
Medium	November – January	4.2	9.8	12.8
Late	February– April	8.3	13.6	–

hatchability. Meanwhile, the females, from the larvae collected in the preheading period of cabbage, laid more eggs, with higher percentage of fertile eggs than those before the preheading period of the plant *(Table 2)*.

Seasonal incidence

Results of field observations indicated that in the north of Vietnam there were 16 population peaks of DBM per year, with durations from peak to peak ranging between 15 to 31 days and the population density at the peaks varied from 0.2 to 24.9 larvae per plant. In general, the peaks with long duration and high density happened in the early part and the end of the year, when there was low temperature and rainfall, and cruciferous vegetables were abundant.

It was found that the duration from peak to peak has close negative correlation with temperature (r = –0.88), but there was no significant correlation with relative humidity and rainfall *(Table 3)*. The peak population density seemed to be negatively correlated with rainfall (r = –0.49).

DBM parasites and their potential

Recently, there have also been attempts made to determine the relative potential of the parasite species in the field. However, there were only 4 species identified; which consisted of: *Trichogramma* sp.,

Table 5: Diamondback moth larval density and date of its peak in different growing periods of cabbage in 1995

Growing time	Peak 1			Peak 2			Peak 3		
	Date	Density/ plant	DAP[1]	Date	Density/ plant	DAP	Date	Density/ plant	DAP
28 Aug.	30/IX	2.8	32	20/X	4.0	52			
3 Aug.	5/X	2.2	32	25/X	5.3	53			
25 Sept.	20/X	1.9	25	15/XI	9.8	51	10/XII	20.1	76
29 Sep.	25/X	1.1	26	20/XI	7.1	52	15/XII	16.7	77
10 Oct.	5/XI	1.7	25	29/XI	9.4	49	25/XII	18.5	76
20 Oct.	15/XI	1.8	25	10/XII	10.2	50	5/I	22.3	76
26 Oct.	20/XI	3.2	25	15/XII	11.8	50	10/I	26.6	
5 Feb.	5/III	9.4	28	30/III	36.0	53			
12 Feb	10/III	10.6	26	5/IV	40.9	52			
21 Feb.	20/III	8.7	27	15/IV	38.8	53			

[1]DAP – Days after planting

Apanteles plutellae Kurdj, Phaeogenes sp., *Beauvaria bassiana*. Among these, *A.plutellae* was the most common in the field and it was able to parasitize DBM population from 1.6–2.1% in early, from 2.2–7.9% in medium and 6.5–10.2% in late growing season of winter.

Problem of cropping time in vegetable area and its effectiveness on the population development of DBM

The results of surveys on cropping system in vegetable areas revealed that the growing time in these areas were undertaken continually, but different in each plot. Because of these, the population abundance of DBM significantly varied between plots.

Generally, in the North of Vietnam, there were 2 peaks of DBM in vegetable plots grown from February to September and 3 peaks in vegetables grown in winter from the end of September to October.

In each plot, the first peak reached 25–32 days after planting (DAP), the second in 49–53 DAP, and the third in 75–77 DAP. These peaks always coincided with the developmental stages of plant, *viz*. leaf development, heading and before harvesting from 10–15 days *(Table 5)*.

The significant aspect found at these peaks was that the proportion of 4th instar larvae was dominant in population with 68–81%. The 1st and 2nd instar larvae always reached the peak at 3–5 days before the occurrence of population peak. It means that the appropriate time for effective control of the pest by insecticides were at 20–27; 45–48 and 70–72 DAP depending on the ambient temperature.

Proposal of programme to control DBM

From the results and literature, a programme to control DBM and other pests in crucifers based on the IPM strategy was proposed. To implement the IPM technology in the crops, the participatory approach is considered to be the norm. The technology to combat DBM is based mainly on 4 components as follows:

- Management of cultural practices in aspects of sanitation, crop rotation, intercropping, fertilizing and watering.
- Routine field observation, combining with manual measures, such as cutting the old leaves, removing egg masses or killing the larvae.
- Insecticide use at the peaks of 1st and 2nd instar larvae of DBM, which means that only 2 or 3 times of insecticide use per crop.
- The effective pesticides to control DBM are products such as *Bacillus thuringiensis* (Bt) Diafenthiuron, and Fipronil used in rotation between biopesticide and chemical pesticides.

The results of IPM technology implementation on cabbage in 1995 have shown the effectiveness in controlling DBM. Pesticide use was reduced by 3–5 sprays, replacing two chemical sprays with Bt. The average costs of control was reduced from 43.3% to 34.6%, and benefits increased from 0.86 to 1.1 million VND per hectare in comparison with farmer's practice.

The diamondback moth: a problem pest of brassica crops in Kenya

G. N. Kibata
Kenya Agricultural Research Institute,
P O Box 57811, Nairobi, Kenya

Abstract

Cruciferous crops are important vegetables in Kenya for local consumption, export and processing (dehydration and oil extraction). They grow well on a wide variety of soils wherever water is available.

One major limiting factor to successful production of these vegetables is the diamondback moth (DBM), *Plutella xylostella* L. (Lepidoptera: Yponomeutidae). The larvae are voracious defoliators with a potential to destroy entire crops if not effectively controlled.

Host resistance, endemic biocontrol agents and cultural practices have not been fully exploited in the management of DBM in Kenya. The easier option has been the use of spurious amounts of chemical pesticides. In the early 1970s, organochlorines such as DDT afforded satisfactory control of DBM. In subsequent years, several carbamate-based products achieved excellent control of the pest. With the advent of synthetic pyrethroids in 1980s, DBM appeared to have been completely subdued. Recent resurgences of the pest and progressive control failure herald intrinsic changes in DBM which make the pest less susceptible to the available chemical arsenal.

This paper reports on pesticide screening trials, undertaken, on farm, to assess efficacy of various products on DBM. It also alludes to circumstantial evidence of pest resistance to popularly used pesticides and is a prognosis for development of more sustainable control strategies.

Key words: DBM, chemical control, Brassica, Kenya.

Introduction

Brassica crops, which include cabbages, kale, brussel sprouts, cauliflower, broccoli and rape seed, are important vegetables in Kenya, largely grown for local consumption, export and processing (dehydration and oil extraction). While most of the production is in the small holder sector, commercial production is on the increase, especially, for cabbage. Available statistics indicate that, collectively, brassicas are grown on over 40,000 hectares with a production of 604,000 metric tonnes, valued at Kenya £104 million. (MALDM, 1988).

The crops grow well on a wide range of soils and agro-ecological zones, wherever water is available. A major production constraint is the diamondback moth (DBM) *Plutella xylostella* L. (Lepidoptera: Yponomeutidae). The larvae of DBM are voracious defoliators which could destroy an entire crop if left uncontrolled. DBM was first recorded as a cosmopolitan agricultural pest in 1746 (Harcourt, 1962). The presence of feeding stimulants (mustard glucosides) and the absence of chemical inhibitors restrict the oligophagous larvae of DBM to the mustard type of plants, the Cruciferae (Thornsteinson, 1953).

It has been recognised that host plant resistance, use of natural enemies and management of the crop agroecology could alleviate problem of DBM (Ullyett, 1947). Several natural enemies of DBM have been recorded in Kenya (Le Pelley, 1959; Kibata, 1978).

The following organisms have been recorded as natural enemies of DBM in Kenya:

Predators

Birds
Spiders
Ants - *Pheidole* sp.
Hover flies *Syritta* sp. and *Melanostoma* sp. (Diptera: Syrphidae)

Parasitoids

Diadegma molliplum (Holmgren)
(= *Stellenboschensis* Cameron)
(Hymenoptera: Ichneumonidae)
Diadegma sp. (Hymenoptera: Ichneumonidae)
Itoplectis melanospilla Cameron (Hymenoptera: Ichneumonidae)
Hemiteles sp. (Hymenopteran: Ichneumonidae)
Diplazon sp. (Hymenoptera: Ichneumonidae)
Brachymeria apantelesi Ribsec (Hymenoptera: Chalcididae)
Pteromalus sp. (Hymenoptera: Pteromalidae)
Tetrastichus sokolowskii Kurdjumov (Hymenoptera: Eulophidae)
Macrogaster sp. (Hymenoptera: Braconidae)
Carducia plutella Emd (Diptera: Tachinidae)

Entomophagous fungi

Possibly *Entomophthora sphaerosperma* Fres. (= *Zoophthora radicans*, Brefeld) has been found to cause epizootics on DBM under favourable conditions. The fungus has potential to control DBM (Wang'endo,

1994). However, given the easier option of using chemical pesticides, little attempt has been made to exploit other pest control strategies.

On a global scale the cost of managing DBM is currently estimated at USD 1 billion through the use of pesticides (Talekar *et al.*, 1992). In South East Asia, the prodigious use of pesticides to control DBM has resulted in the typical pesticide treadmill where shifting and changing of chemicals no longer solves the problem.

In Kenya chemical control of DBM was successfully undertaken with organochlorine insecticides, such as DDT, lindane and endosulfan, in 1970s. In later years carbamates became more popular, such as carbaryl, cartap hydrochloride, acephate and carbofuran. With the advent of synthetic pyrethroids in 1980s, DBM became a problem of the past, as dramatic control of the pest was achieved (Kibata, 1978).

Recently, however, the scencario has been gradually changing as more intensive production of brassicas and rampant usage of pyrethroids continues. Reports on control failure for DBM were on the increase, prompting further search for products with varied modes of action. This paper reports on recent trials undertaken to evaluate new and old products for this purpose. There is, however, no claim that the DBM problem is resolved.

Materials and Methods

Four series of trials were conducted at two farms in the north coast of Naivasha (0° 43' 40"S, 36° 24', 32"N, 1921m asl) from April 1994 to May 1995. The trials design was CRBD with three replicates, whilst treatments ranged from 19 to 21 entries, inclusive of untreated checks (controls).

Seedlings of cabbage (*Brassica oleracea* var. *capitata*) variety Gloria were transplanted into plots at row and plant spacing of 60 cm and 30 cm respectively. Inter block and inter plot paths were 2 m and 1m, respectively.

Plant stand counts for the four trials were 60, 130, 99 and 105 per plot, respectively, exempting guard rows. Fertilizer was applied as recommended for cabbage at transplanting and subsequently as top dressings.

Once the seedlings were established, weekly samplings for immature DBM were undertaken on five cabbages per treatment plot. Application of test insecticides were made fortnightly using a CP3 knapsack sprayer with a hollow cone nozzle calibrated to deliver 400 litres of spray per hectare.

At maturity all marketable cabbage heads were harvested, counted and weighed for assessment of yield. The pooled means of DBM counts for immatures over the crop season, numbers of marketable cabbage heads and yield was subjected to ANOVA and DMRT to assess effects of treatments on these parameters *(Tables 1, 2, 3, 4)*.

Results

The collective results of the four trials demonstrated that there are significant differences between treatments (p <0.05) with regard to DBM infestation, numbers of marketable cabbage heads and related yield *(Tables 1, 2, 3, & 4)*. Whilst the DBM infestation varied between the trials, higher infestation was observed in first and fourth trial runs. The trend was, however, consistent with respect to efficacious products in comparison with untreated control plots. Mean DBM infestation was highest in control plots where yield of marketable cabbage was also lowest in all instances.

Effective products more than doubled the yield of cabbage, which confirms the need to evolve effective management strategies for DBM.

On the basis of DBM suppression and cabbage yield the test products could be classified as follows:

Very effective

- Secure® (AC 303630) - Insecticide, acaricide trifluoromethyl pyrrole carbonitrile
- Fipronil - phenyl pyrazole insecticide, acaricide
- Prothiofos (Tokuthion®) - organophosphate (OP)
- BtXentari® - *Bacillus thuringiensis*, Berliner subsp. *aizawai*
- Novaluron® (MCW-275) - Benzoylphenyl urea (IGR)

Effective

- Methidathion - organophosphate
- Methomyl + Methidathion - carbamate/OP
- Methomyl - carbamate

Least effective

- Methamidophos - organophosphate
- Cypermethrin - pyrethroid
- Acephate - carbamate
- Fenpropathrin - pyrethroid
- Neem powder - Botanical
- Etofenprox (Trebon®) - non-ester pyrethroid

Discussion

Results of these trials subscribe to the view that pyrethroids which have been extensively used are no longer as effective as they used to be whilst products from new chemistry appeared extremely effective against DBM.

Among the most effective products, Secure® (AC 303630) is an insecticide and acaricide from Cyanamid based on 4-bromo-2-(4-chloropheny)-1-ethoxymethyl-5-trifluoromethylpyrrole-3=carbonitrile. Fipronil is a phenyl pyrazole insecticide acaricide from Rhone-Poulenc. Prothiofos is an old OP insecticide from Bayer while Bt Xentari is a new insecticide based on *Bacillus thuringiensis*, Berliner subspecies *aizawai* from the same company. Novaluron (MCW-275) is a novel benzoylphenyl urea from Makhteshim Agan. Evidently, the modes of action of these products are

Table 1. Effect of treatments on DBM infestation and cabbage yield at Aberdare farm, (Trial period 20/04/94 to 01/06/94) (plant population 60)[1]

Treatments	Rate g.a.i. ha^{-1}	Mean DBM counts	Mean marketable heads	Mean cabbage yield (kg) per plot
1. Untreated	–	17.01_{a}	9.7_{a}	5_{a}
2. Acephate 750WP	600	6.67_{fg}	33_{def}	36_{fg}
3. Fipronil 50 SC	12.5	5.87_{gh}	35_{fg}	46_{hij}
4. Fipronil 50 SC	25	4.81_{h}	42_{g}	54_{ij}
5. Fipronil 50 SC	50	2.05_{i}	50_{h}	75_{k}
6. Etofenprox 200 Ec	100	11.02_{bc}	16_{ab}	11_{ab}
7. Chlorpyrifos 480 Ec	384	11.40_{b}	20_{bc}	14_{bc}
8. Dichlorvos 500 Ec	400	10.10_{cd}	20_{bc}	17_{bc}
9. Thiodicarb 375 Ec	562.5	8.69_{e}	23_{bcd}	19_{bcd}
10. Methomyl 900 SP	360	7.34_{f}	33_{ef}	36_{fg}
11. "Secure" 360 SC[2]	144	1.50_{i}	42_{g}	71_{k}
12. Cypermethrin 50 Ec	50	8.61_{e}	29_{cdef}	32_{efg}
13. Methidathion 400 Ec	240	5.88_{gh}	28_{cdef}	31_{ef}
14. Methidathion 200 + Methomyl 50 Ec	150	9.11_{de}	31_{def}	37_{fgh}
15. Methidathion 200 + Methomyl 50 Ec	200	5.02_{h}	32_{def}	41_{ghi}
16. Pyrethrins 60 Ec	36	12.20_{b}	28_{cdef}	34_{efg}
17. Bt Xentari[3]	1000g	4.75_{h}	33_{f}	50_{ij}
18. Methamidophos 600 SC	360	7.28_{f}	24_{bcde}	27_{de}
19. Methidathion 200 + Methomyl 50 Ec	300	7.29_{f}	23_{bcd}	20_{cd}

[1]Means followed by the same letter within the same column are not significantly different (P > 0.05) by DMRT
[2]Secure" proposed common name for AC 303630
[3]Bt Xentari $15x10^3$ $I.U.mg^{-1}$ (*Bacillus thuringiensis* subsp. *aizawai*) 1000g of product applied per hectare

Table 2. Effect of treatments on DBM infestation and cabbage yield at Aberdare farm, (Trial period 06/06/94 to 17/08/94 (plant population 130)[1]

Treatments	Rate g.a.i/ha	Mean DBM counts	Mean marketable heads	Mean cabbage Yield (kg) per plot
1. Untreated	–	2.80_{a}	62.7_{a}	144_{a}
2. Acephate 750 WP	600	0.89_{ef}	83_{ab}	242_{cd}
3. Fipronil 50 SC	12.5	0.40_{hi}	97_{bc}	280_{cde}
4. Fipronil 50 SC	25.0	0.37_{hij}	127_{def}	342_{efg}
5. Fipronil 50 SC	50.0	0.27_{hij}	129_{def}	387_{efg}
6. Etofenprox 200 Ec	200	1.85_{b}	63_{a}	152_{ab}
7. Prothiofos 960 Ec	768	0.25_{hij}	128_{def}	410_{gh}
8. Azadirachtin[2]	1.5 L	1.65_{c}	66_{a}	152_{ab}
9. Thiodicarb 375 Ec	562.5	1.92_{b}	63_{a}	157_{ab}
10. Methomyl 900 SP	360	1.08_{d}	82_{ab}	229_{bc}
11. "Secure" 360 SC[3]	144	0.21_{i}	127_{def}	445_{h}
12. Cypermethrin 50 Ec	50	1.12_{d}	83_{ab}	216_{bc}
13. Methidathion 400 Ec	20	0.67_{g}	99_{bcd}	248_{cd}
14. Methidathion 200 + Methomyl 50 Ec	150	0.44_{h}	100_{bcde}	300_{de}
15. -ditto-	200	0.41_{hi}	102_{bcdef}	305_{de}
16. -ditto-	300	0.24_{ij}	112_{cdef}	335_{ef}
17. Azadirachtin Ec[4]	2 g/plant	0.76_{fg}	89_{abc}	240_{cd}
18. Bt Xentari[5]	1 000	0.22_{ij}	127_{def}	405_{gh}
19. Methamidophos 600 Sc	600	1.02_{de}	75_{ab}	217_{bc}

[1]Means followed by the same letter within the same column are not significantly different (P > 0.05) by DMRT.
[2]Neem seed liquid extract (JAWAN®) from India applied at 1.5 litres per hectare.
[3]"Secure" proposed common name for AC 303630.
[4]Neem seed powder (locally produced) 2% a.i. applied at 2g/plant.
[5]Bt (Xentari) $15x10^3$ $I.U.\ mg^{-1}$ (B.t. subsp. *aizawai*) applied 1000g/ha.

different from that of pyrethroids which are relatively ineffective on DBM at Naivasha.

From results of the last trial it was shown that DBM infestation was significantly related (P < 0.001) with numbers of marketable cabbage heads ($r^2 = 0.69$) (Y = 87.99 – 7.013x) as well as the marketable weight of cabbage ($r^2 = 0.61$) (Y = 236.91 – 0.198x). This is an expected outcome as heavily infested cabbage failed to form heads or formed heads which could not be marketed.

It is therefore pertinent to conclude that DBM causes substantial economic loss in cabbage

Table 3. Effect of treatments on DBM infestation and cabbage yield at Boffar farm, Naivasha, (Trial period 31/08/94 to 14/10/94) (plant population 99)[1]

Treatments	Rate g.a.i./ha^{-1}	Mean DBM counts	Mean marketable heads	Mean cabbage yield (kg) per plot
1. Untreated	–	2.83_{a}	49_{a}	39_{abc}
2. Acephate 750 WP	600	0.96_{d}	57_{abc}	68_{cd}
3. Fipronil 50 SC	12.5	0.63_{fg}	58_{abc}	104_{gh}
4. Fipronil 50 SC	25.0	0.46_{hi}	67_{e}	106_{gh}
5. Fipronil 50 SC	50.0	0.25_{j}	70_{cd}	105_{gh}
6. Etofenprox 200 Ec	200	1.55_{b}	50_{ab}	25_{a}
7. Prothiofos 960 Ec	768	0.28_{j}	90_{e}	180_{j}
8. Azadirachtin[2]	1.5 L	1.36_{c}	52_{ab}	58_{bc}
9. Thiodicarb 375 Ec	562.5	1.35_{c}	51_{ab}	56_{bc}
10. Methomyl 900 SP	360	0.97_{d}	57_{abc}	68_{cd}
11. “Secure” 360 SC[3]	144	0.31_{ij}	87_{e}	130_{i}
12. Cypermethrin 50 Ec	50	0.87_{de}	65_{bc}	91_{fg}
13. Methidathion 400 Ec	320	0.67_{fg}	59_{abc}	90_{fg}
14. Methidathion 200 + Methomyl 50 Ec	300	0.57_{gh}	61_{abc}	60_{c}
15. Novaluron 100 Ec	5	0.75_{ef}	60_{abc}	83_{def}
16. Novaluron 100 Ec	10	0.57_{gh}	58_{abc}	82_{def}
17. Azadirachtin[4]	2g/plant	0.87_{de}	58_{abc}	93_{fgh}
18. Bt Xentari[5]	1000g	0.56_{gh}	80_{de}	112_{hi}
19. Methamidophos 600 SC	600	0.59_{fgh}	58_{abc}	69_{cde}
20. Bt Xentari[6]	500g	0.99_{d}	63_{abc}	89_{efg}

[1]Means followed by the same letter, in the columns, are not significantly different (P > 0.05) by DMRT.
[2]Neem seed liquid extract (JAWAN®) from India applied 1.5 litres ha^{-1}
[3]“Secure” proposed common name for AC 303630.
[4]Neem seed powder (locally produced) applied at 2g/plant.
[5&6]Bt (Xentari) 15x10^{3} I.U. mg^{-1} B.t. subsp. *aizawai* applied at 500g and 1000g product ha$^{-1.}$

Table 4. Effect of treatments on DBM infestation and cabbage yield at Aberdare farm, (Trial period 05/04/95 to 17/05/95) (plant population 105)[1]

Treatments	Rate g.a.i./ha^{-1}	Mean DBM counts	Mean marketable heads	Mean cabbage yield (kg) per plot
1. Untreated	–	18.39_{a}	2.00_{h}	0.60_{g}
2. Acephate 970[2]	776	9.73_{ef}	2.33_{h}	1.47_{g}
3. Fipronil 50 SC	12.5	6.91_{g}	49.00_{de}	99.67_{def}
4. Fipronil 50 SC	25.0	5.00_{ij}	66.00_{c}	148.60_{cd}
5. Fipronil 50 Sc	50.0	3.55_{k}	90.00_{ab}	269.70_{ab}
6. Prothiofos 960 Ec	768	10.58_{de}	3.67_{h}	2.70_{g}
7. “Secure” 360 SC[3]	144	2.30_{l}	94.67_{a}	320.70_{a}
8. Cypermethrin 50 Ec	50	13.64_{b}	0.67_{h}	0.13_{g}
9. Methidathion 400 Ec	320	5.79_{hi}	39.33_{efg}	65.33_{defg}
10. Methidathion 200 + Methomyl 50 Ec	300	6.09_{gh}	29.67_{fg}	53.67_{efg}
11. Novaluron 100 Ec	5	5.97_{h}	34.00_{fg}	93.17_{def}
12. Novaluron 100 Ec	10	4.76_{i}	51.67_{d}	143.80_{cde}
13. Bt Xentari[4]	500g	5.61_{hij}	40.67_{ef}	98.53_{def}
14. Bt Xentari	1000g	3.79_{k}	81.67_{b}	217.30_{bc}
15. Methamidophos 600 Sc	600	6.94_{g}	28.67g	47.17_{fg}
16. Triflumuron 480 SC	240	10.67_{dc}	2.33_{h}	2.53_{g}
17. Fenpropathrin 100 Ec	100	9.97_{e}	1.00_{h}	0.43_{g}
18. Fenpropathrin 100 Ec	50	11.30_{cd}	0.33_{h}	0.17_{g}
19. Beta-cyfluthrin 25 Ec	15	8.88_{f}	0.67_{h}	0.47_{g}
20. Endosulfan 475 FL	475	11.61_{c}	1.00_{h}	0.37_{g}
21. Carbosulfan 250 Ec	250	10.58_{de}	0.33_{h}	0.17_{g}

[1]Means followed by the same letter within columns, are not significantly different (P > 0.05) by DMRT.
[2]Acephate (97% pellets)
[3]“Secure” proposed common name for AC 303630
[4]Bt (Xentari) 15x10^{3} I.U. mg^{-1} Bt subs. *aizawai* applied at 500 and 1000g ha^{-1}

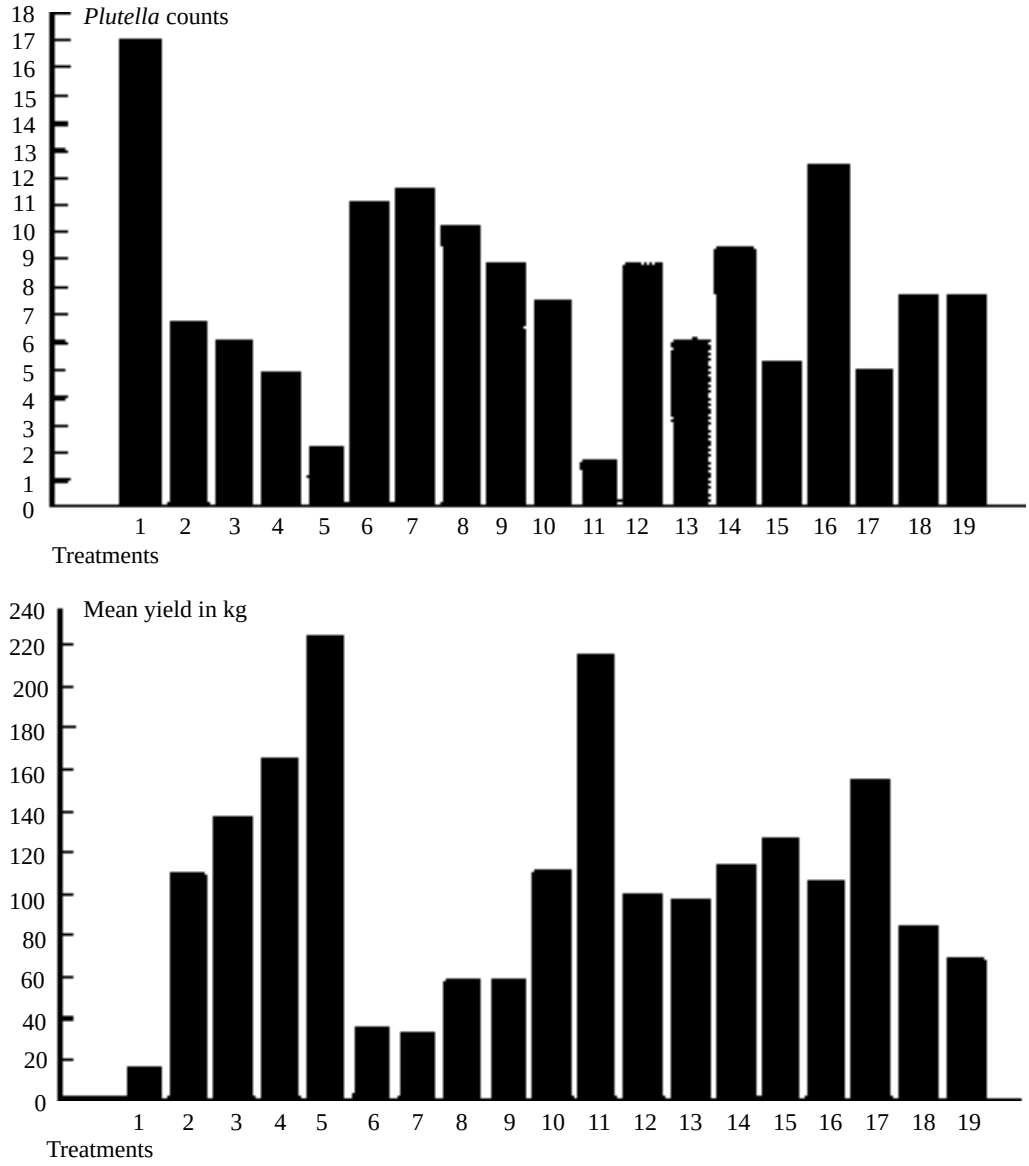


Figure 1. Mean counts of DBM and yield in various treatments (refer table 1) in Trial 1

production. Some commercially available pesticides are no longer affording the level of desired control on DBM. Products under development, whose mode of action is novel, appear to be extremely effective on DBM. This heralds a warning that pest resistance to popular insecticides may have developed in the diamondback moth. The need to develop a comprehensive strategy for better management of DBM is therefore advocated if successful production of Brassica crops is to be sustained in Kenya.

It is also noted that previous work on identification of other major mortality factors for DBM identified several natural enemies. These were only collected from farms where no pesticides were being used or where there were adequate refugia in wild cruciferous weeds or neglected Brassica crops. The natural enemies did not however appear to confer satisfactory suppression of DBM on their own. An integrated approach to the management of DBM should therefore be explored.

Acknowledgement

The author wishes to gratefully acknowledge the assistance of Messrs S. O. Pete and J. W. Kamau who assisted in the implementation of the trials and Margaret Gitonga who diligently typed the manuscript. The work would have been impossible without the financial and moral support of the KARI/ODA Crop Protection Project. Preparation of this paper has been duly authorised by the Director, Kenya Agricultural Research Institute (KARI). The author is gratefully indebted to the GTZ-IPM Horticulture project for the sponsorship to this conference.

References

Harcourt, D.G. (1962). Biology of cabbage caterpillars in Eastern Ontario. *Proc. Entomol. Soc. Ont.* ***93:*** 61–65.

Kibata, G.N. (1978). Control of Diamondback moth caterpillars on cabbage p. 15–18. Senior Entomologist's Annual Report. 60pp.

Le Pelley, R.H. 1959. Index of insect hosts of parasites: *Plutella maculipennis* - Plutellidae p.245. In Agricultural Insects of East Africa. E.A. High Comm. Nairobi, Kenya 307pp.

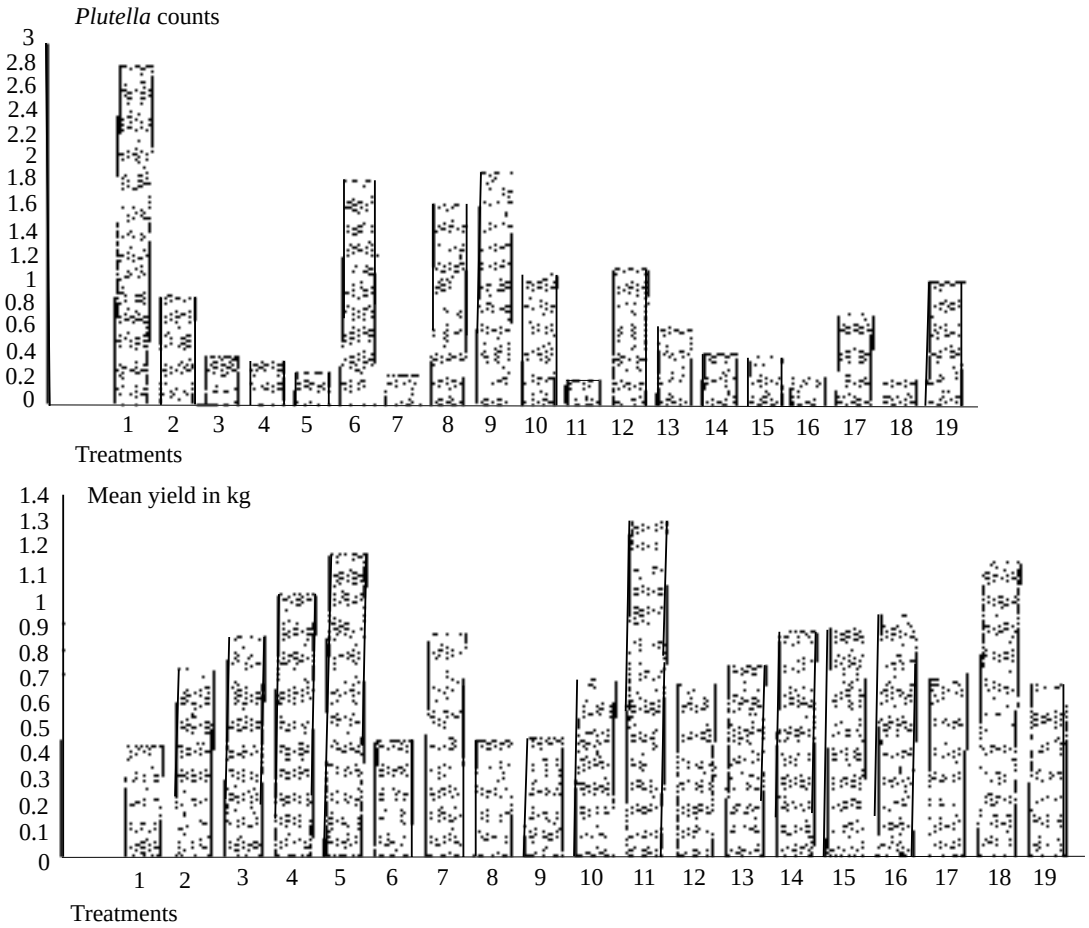


Figure 2. Mean counts of DBM and yield in various treatments (refer table 2) in Trial 2

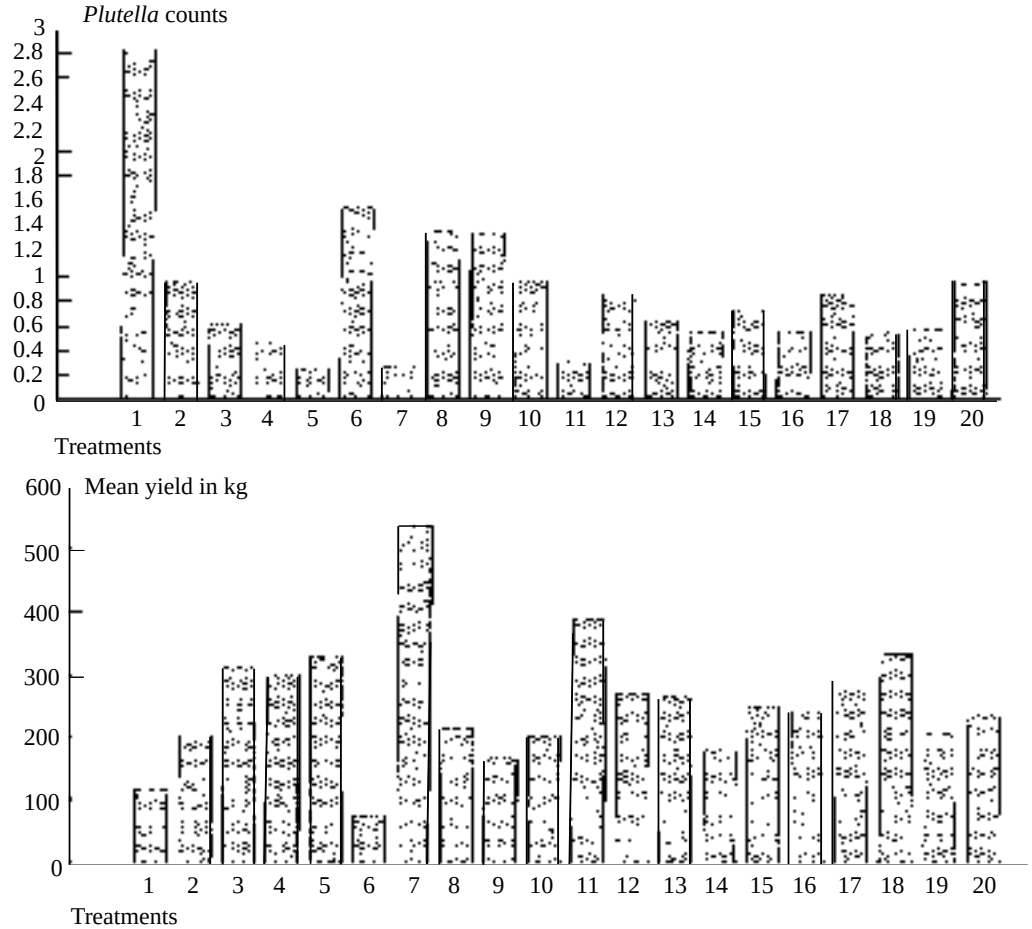


Figure 3. Mean counts of DBM and yield in various treatments (refer table 3) in Trial 3

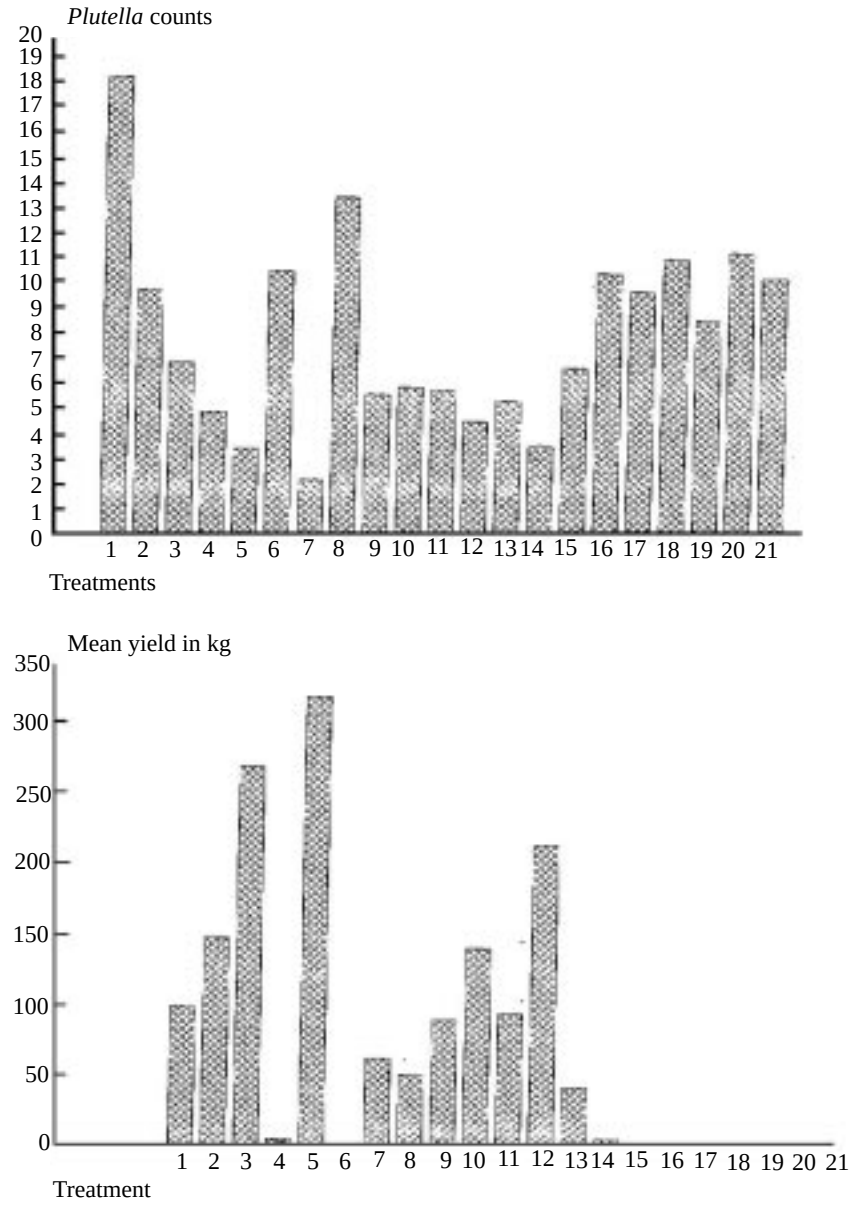


Figure 4. Mean counts of DBM and yield in various treatments (refer table 4) in Trial 4

MALDM (1988). Crop Production statistics. Min. of Agric. Liv. Dev & M. Kenya.

Talekar, N.S., J.C. Yang, and S.T. Lee, (1992). Introduction of *Diadegma semiclausum* to control diamondback moth in Taiwan. In "Diamondback moth and other crucifer pests, Proceedings of the Second International Workshop, Tainan, Taiwan. Talekar, N.S. (ed.), pp. 263–270. AVRDC.

Thorsteinson, A.J. (1953). The chemotactic reponses that determine host specificity in an oligophagus insect (*Plutella maculipennis* (Curt) Lepidoptera). *Can. J. Zool.* ***31:*** 52–72.

Ullyett, G.C. (1947). Mortality factors in populations of *Plutella maculipennis* Curtis and their relation to the problem of control. *Entomol. Mem. Dept. Agr. S. Africa.* ***2:*** 77–202.

Wang'endo, L. (1994). Interactions between the insect growth regulator, Flufenoxuron (CASCADE$^{R;}$); the entomopathogen, *Zoophthora radicans* Brefeld and the Diamondback moth *Plutella xylostella* L. MSC thesis, Univ. of Bristol, United Kingdom.

The diamondback moth with special reference to its parasitoids in South Africa

Rami Kfir

Plant Protection Research Institute, Private Bag X134, Pretoria 0001, South Africa

Abstract

The pest status of diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), in South Africa is lower than in other countries with similar climates. A project was initiated to investigate possible reasons for this. Larval and pupal populations of DBM were monitored weekly for two years on unsprayed cabbage plots. Adult populations were also monitored continuously for two years using synthetic sex-pheromone traps. Samples of DBM were taken to the laboratory and parasitoids that emerged were identified and their incidence determined. Moths, larvae and parasitoids were active throughout the year. Infestations were low from January to September and high during October to December. Even when infestations in the field were low, a high percentage of plants was infested, indicating a regular distribution of DBM in the sample plots. In general, parasitism of DBM larvae and pupae was high (reaching 90–100%) except in the winter months of June-August when it was low. Twenty one species of parasitoids and hyperparasitoids were reared from DBM larvae and pupae: the egg-larval parasitoids *Chelonus curvimaculatus* Cameron and *Chelonus* sp. (Braconidae); the larval parasitoids *Apanteles eriophyes* Nixon, *Cotesia plutellae* (Kurdjumov), *Habrobracon brevicornis* (Wesmael) (Braconidae) and *Peribaea* sp. (Tachinidae); the larval-pupal parasitoids *Diadegma* sp., *Itoplectis* sp. (Ichneumonidae) and *Oomyzus sokolowskii* (Kurdjumov) (Eulophidae); the pupal parasitoids *Brachymeria* sp., *Hockeria* sp. (Chalcididae), *Diadromus collaris* Gravenhorst (Ichneumonidae) and *Tetrastichus howardi* (Olliff) (Eulophidae); and the hyperparasitoids *Aphanogmus fijiensis* (Ferrière) (Ceraphronidae), *Brachymeria* sp., *Hockeria* sp, *Proconura* sp. (Chalcididae), *Mesochorus* sp. (Ichneumonidae), *Pteromalus* sp. (Pteromalidae), *Eurytoma* sp. (Eurytomidae) and *Tetrastichus* sp. (Eulophidae). The large number of indigenous plants from the Brassicaceae, the many species of DBM parasitoids and a bisexual form of the parasitoid *D. collaris* in South Africa suggest that DBM might have originated in southern Africa.

Key words: *Plutella xylostella*, biological control, parasitoid, South Africa

Introduction

Cabbage, *Brassica oleracea* var. *capitata*, is cultivated throughout the year in most parts of southern Africa since heat-tolerant cultivars have become available (Hemy, 1984). In South Africa, cruciferous crops are grown commercially near markets in urban areas for economic reasons. Commercial cabbage is grown on *ca*. 9 000 ha with an annual production of 450 000 tons and cauliflower on 2 000 ha yielding 40 000 tons. Rapeseed, *Brassica napus*, a new crop to South Africa, was introduced in 1994. During the first year 5 000 ha were planted, 15 000 ha were planted in 1995 and the projection for 1998 is 100 000 ha. Small amounts of other brassicas are also produced annually.

Diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) is an important pest of cruciferous crops in South Africa (Annecke and Moran, 1982). Its biology was studied by Gunn (1917) and the natural mortality factors by Ullyett (1947), in South Africa. Eleven parasitoids, a few predators and the fungus, *Entomophthora sphaerosperma* Fres, where identified as natural enemies of DBM in South Africa by Ullyett (1947). The taxonomy of these organisms was not well known at that time and it is in need of revision. Moreover, the specimens on which the work was based have been lost as they could not be traced in the National Collection of Insects, Plant Protection Research Institute, Pretoria or in the Natural History Museum, London. Except for the superficial study by Dennill and Pretorius (1995) in which only one parasitoid of DBM was recorded, no further studies on the natural enemies of DBM have been undertaken in South Africa since Ullyett's work in the Pretoria area during the 1930's.

In South Africa the pest status of DBM is lower than in other countries with similar climates. Because of indiscriminate use of pesticides against the pest by farmers, local populations of DBM started to show signs of resistance to insecticides and its pest status is increasing. It is also expected that the rapidly increasing cultivation of rapeseed may lead to heavy attacks by DBM, which could necessitate aerial applications of chemical pesticides against the pest in the long run. This would undoubtly result in the acceleration of resistance development. The implications of this are serious because the consumption of cruciferous crops in South Africa is increasing, particularly in disadvantaged communities of rural and peri-urban areas where cabbage is a staple vegetable.

The study reported here was undertaken to update the information on DBM and its natural enemies in

South Africa. The results will contribute to the development of an IPM programme for cruciferous crops before complete resistance of DBM to chemical pesticides has developed.

Material and Methods

Larval, pupal and moth populations of DBM were monitored each week for two years in cabbage fields (three consecutive cabbage crops were planted each year) at Hartebeespoort Agricultural Research Station near Brits (25 °38'S, 27 °47'E; altitude 1102m), North-West Province, South Africa.

Synthetic sex-pheromone traps were deployed to monitor the flight pattern of DBM. Three delta-shaped traps (registered trade name 'Biotrap') were used. In the traps, sticky floors coated with a layer of polybutene adhesive were used. The sticky floors were replaced weekly when the traps were examined and moth catches recorded. The pheromone dispensers were placed in the middle of the sticky floor within the metal trap (26 X 9.5 X 13 cm high).

Standard cultivation practices were used in the cabbage fields, including weeding, fertilizing and irrigation. Infestation of the plants with DBM was allowed to take place and no control measures were applied. At weekly intervals, 30 plants were selected randomly, inspected and the numbers of DBM larvae, pupae and parasitoid cocoons were recorded for each plant. Samples of DBM larvae, pupae and parasitoid cocoons were collected. In the laboratory, the pupae and the parasitoid cocoons were transferred singly into glass vials (2.5 X 10 cm). The larvae were provided with sections of fresh cabbage leaves and held singly in Petri dishes. The leaves were replaced every second day until the larvae pupated or parasitoid cocoons were formed. The collected insects were held in the laboratory at 22 $\pm$ 2 °C, 60 $\pm$ 5% RH, and pupation of larvae and emergence of moths was recorded, as well as parasitoid emergence. All parasitoids were identified and their incidence and relative abundance were calculated. Larvae that escaped or died of unknown causes were excluded from the calculation of parasitism rates.

Results

The moths collected in the pheromone traps indicated that DBM was active throughout the year. Numbers of moths were low, less than 5 moths per trap, from January to August, but much higher during September to December, peaking at about 25 and 100 moths per trap in 1993 and 1994, respectively, in the spring (September to October), *(Fig. 1)*.

The flight activity of the moths corresponded with larval infestation on the crops. Larval infestations and crop damage were relatively low from January to August with numbers fluctuating between zero and four larvae per plant. The larval infestation increased in spring and peaked at 18 and 74 larvae per plant in 1993 and 1994, respectively, during the September-October period *(Fig. 2)*. The distribution of the pest on the crops was regular, as indicated by the high proportion of infested plants, even at low infestation levels *(Fig. 2)*.

The DBM parasitoids were also active throughout the year with parasitism levels of larvae and pupae peaking at 90–100% on several occasions *(Fig. 3)*. After two years of sampling, 21 species of parasitoids and hyperparasitoids were identified.

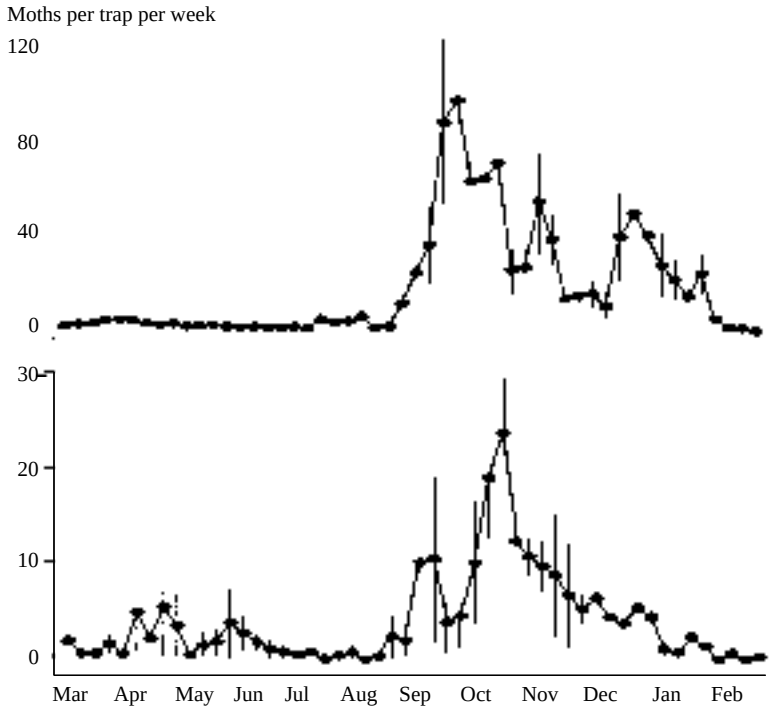


Fig. 1. Synthetic sex-pheromone trap catches of diamondback moth during 1993 (below) and 1994 (above) at Brits. Bars represent standard errors (SE) when larger than symbol size

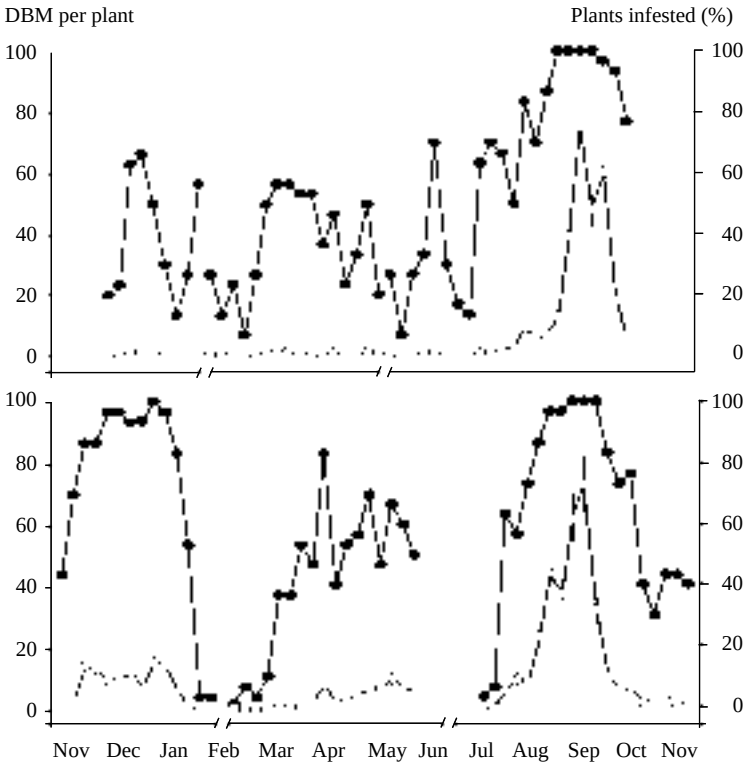


Fig. 2. Abundance of diamondback moth during 1993 (below) and 1994 (above) in unsprayed cabbage at Brits (three cabbage crops per year). Proportion of plants infested (solid circles); number of larvae per plant (open circles). Bars represent standard errors (SE) when larger than symbol size

Egg-larval parasitoids

Chelonus curvimaculatus (Cameron) (Braconidae). This rare solitary endoparasitoid occurred during February to May. The parasitoid female oviposits in DBM eggs and the parasitoid larva emerges from a fully grown DBM larva to spin its cocoon. The species is very variable and several species are probably involved. *C. curvimaculatus* is a polyphagous parasitoid that has also been recorded from the following hosts in South Africa: the spotted stem borer, *Chilo partellus* (Swinhoe) (Kfir, 1992); the African stem borer, *Busseola fusca* (Fuller) (Kfir, 1995); the potato tuber moth, *Phthorimaea operculella* (Zeller) and the Karoo caterpillar, *Loxostege frustalis* (Zeller) (Broodryk, 1969).

Chelonus sp. (Braconidae). This is a rare parasitoid of DBM with a similar biology to *C. curvimaculatus*.

Larval parasitoids

Apanteles eriophyes (Nixon) (Braconidae). This is an abundant solitary endoparasitoid of DBM in South Africa. Its activity ceased only during the cold winter months (June to August). *A. eriophyes* is specific to DBM, is restricted to South Africa and may be the same as *Apanteles halfordi* Ullyett (Walker and Fitton, 1992). In the absence of the type specimens of *A. halfordi*, Walker and Fitton (1992) recommended to use the name *A. eriophyes* for this parasitoid.

Cotesia plutellae (Kurdjumov) (Braconidae). This solitary endoparasitoid was the most abundant parasitoid during the current study, occurring throughout the year. In South Africa, several distinct yellow colour patterns appear on the third abdominal tergite of this species. This colour patterns appear to be influenced by the season. *C. plutellae* has been widely used in biological control programmes against DBM and was introduced into many countries (Lim, 1986, 1992; Fitton and Walker, 1992).

Habrobracon brevicornis (Wesmael) (Braconidae). This is a rare, gregarious ectoparasitoid with a wide host range. In this study only one parasitoid completed development on a 4th instar DBM. Specimens were collected during May to July. *H. brevicornis*, which is regarded as a synonym of *H. hebetor* (Say), is widely distributed in the Afrotropical, Nearctic, Neotropical, Oriental and Palaearctic Regions (Van den Berg *et al*., 1988). Its biology in South Africa was reported by Taylor (1932).

Peribaea sp. (Diptera: Tachinidae). This rare parasitic fly was recorded from DBM larvae during winter.

Larval-pupal parasitoids

Diadegma sp. (Ichneumonidae). This solitary endoparasitoid was abundant during most of the year except in the cold winter months (June to August). It is apparently an undescribed species (M. Fitton, personal communication).

Itoplectis sp. (Ichneumonidae), is a rare solitary endoparasitoid of DBM in South Africa.

Oomyzus sokolowskii (Kurdjumov) (Eulophidae). This facultative hyperparasitoid is an abundant, gregarious parasitoid of DBM. *O. sokolowskii* occurs

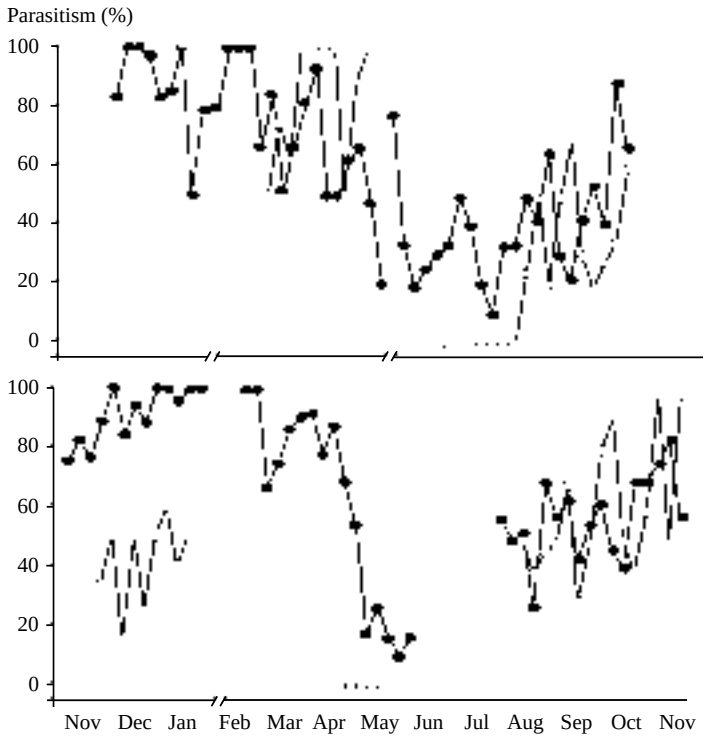


Fig. 3. Percentage parasitism of diamondback moth larvae (solid circles) and pupae (open circles) on unsprayed cabbage during 1993 (below) and 1994 (above) at Brits (three cabbage crops per year)

in Europe, Asia and Africa (Harcourt, 1960). Its biology was studied in Malaysia by Ooi (1988) and in Pakistan by Mushtaque (1990). *O. sokolowskii* has been introduced into many countries for biological control of DBM (Lim, 1992), and following its introduction and establishment in the Cape Verde Islands, together with *C. plutellae*, brought about the control of the pest (Cock, 1983). In South Africa, up to 20 individuals may complete development in one host but normally only about 8–10 individuals develop. *O. sokolowskii* only parasitises the larvae of DBM and normally emerges from the pupae. On several occasions parasitised DBM larvae became mummified before pupation and the parasitoids emerged from them. In rare cases, *O. sokolowskii* also emerged from cocoons of *C. plutellae* and can only become a hyperparasitoid when it parasitises DBM larvae which were previously parasitised by *C. plutellae*. Its activity as a primary parasitoid far exceeds its hyperparasitic activity, and *O. sokolowskii* should consequently be considered as a valuable natural enemy of DBM.

Pupal parasitoids

Brachymeria sp. (Chalcididae). Another rare parasitoid that was only active in summer (October to January).

Diadromus collaris (Gravenhorst) (Ichneumonidae). This parasitoid was very common during autumn (April and May) and spring (August and September). In South Africa, *D. collaris* reproduces sexually, as males were abundant in field populations. In Europe, on the other hand, it is uniparental with only females known (M. Fitton, personal communication; R. Zwart, personal communication). *D. collaris* has been widely used in biological control projects against DBM. It was introduced from England and became established in New Zealand (Hardy, 1938) and Australia (Wilson, 1960). It was also introduced into the Cameron Highlands of Malaysia (Ooi and Lim, 1989) where it became a dominant parasitoid, together with *C. plutellae* and *Diadegma semiclausum* Horstmann (Syed *et al*., 1990).

Hockeria sp. (Chalcididae). This rare parasitoid was active only in summer.

Tetrastichus howardi (Olliff) (Eulophidae). Also known as *T. ayyari* and *T. israeli* (Fitton and Walker, 1992). This parasitoid is distributed through Pakistan, Mauritius, Taiwan and eastern Australia (Boucek, 1988). Prior to 1977, *T. howardi* was the only parasitoid of DBM present in the Cameron Highlands of Malaysia, a major vegetable-producing area, before introductions of other DBM parasitoids were made (Talekar and Shelton, 1993). *T. howardi* was introduced into South Africa from the Philippines for the biological control of cereal stem borers and was recorded several times from pupae of DBM. Its biology and host preference in South Africa was studied by Kfir *et al*.(1993) and Moore & Kfir (1995a, 1995b).

Hyperparasitoids

During the current study, hyperparasitoids were very active during spring (September to October) when DBM populations were high and primary parasitoids abundant. In this period the majority of parasitoids emerging from some field samples of parasitoid cocoons and DBM larvae were hyperparasitoids. Some samples yielded hyperparasitoids only. During the rest of the year hyperparasitoids were relatively rare. The

most abundant hyperparasitoids were the solitary endoparasitoids *Pteromalus* sp. and *Mesochorus* sp.

Mesochorus sp. (Ichneumonidae) attacked larvae of *C. plutellae* and *A. eriophyes* inside the DBM larvae. *Mesochorus* sp. only started to feed after the primary parasitoids had completed their development and formed cocoons. *Mesochorus* sp. pupated inside the cocoon of its host and then emerged from it.

Pteromalus sp. (Pteromalidae) attacked cocoons of *C. plutellae*, *A. eriophyes* and *Diadegma* sp. Occasionally *Pteromalus* sp. also attacked *D. collaris* inside pupae of DBM.

Aphanogmus fijiensis (Ferrière) (Ceraphronidae). This gregarious, polyphagous hyperparasitoid was reared from field samples of *C. plutellae* and *A. eriophyes* cocoons on several occasions. Between 4 to 7 *A. fijiensis* completed development in each cocoon. In South Africa, *A. fijiensis* was also recorded from cocoons of *Cotesia sesamiae* (Cameron) (Braconidae), a parasitoid of *C. partellus* and *B. fusca*, and from *Cotesia kazak* (Telenga), an introduced parasitoid of *Helicoverpa armigera* Hübner (Kfir, 1990; 1995). *A. fijiensis* was recorded as a hyperparasitoid of DBM when it was found attacking *C. plutellae* in the Philippines (Poelking, 1992) and on several Caribbean islands (Cock, 1985; Alam, 1992).

Other hyperparasitoids that occasionally emerged from cocoons of *C. plutellae* and *A. eriophyes* were the solitary *Eurytoma* sp. (Eurytomidae), *Tetrastichus* sp. (Eulophidae), *Hockeria* sp., *Brachymeria* sp. and *Proconura* sp. (Chalcididae).

Discussion

The fauna of DBM parasitoids in South Africa was found to be rich. The 21 species of parasitoids and hyperparasitoids reared from DBM larvae and pupae in South Africa, some specific to DBM and known only from South Africa, indicate a very long association between parasitoids and the pest in the region. By contrast, only few indigenous, non-specific parasitoids were recorded from DBM in many countries where DBM is exotic. In New Zealand, for example, only two parasitoids, *Diadegma laterallis* (Grav) and *Diadromus* sp., which were responsible for only 7% parasitism, were recorded before the introduction of exotic parasitoids (Muggeridge, 1930; Robertson, 1939). Similarly, in Malaysia, only *T. howardi*, was present but it had a negligible effect on DBM populations (Talekar and Shelton, 1993).

DBM feeds only on plants belonging to the family Brassicaceae (Talekar and Shelton, 1993). It is reasonable to assume that DBM evolved on plants from this family. Because cultivated brassicas are considered to be of European origin, it was suggested by Hardy (1938) and since then widely accepted that DBM had also originated in the same area, probably the Mediterranean region. A total of 175 wild plant species in the Brassicaceae have been recorded in South Africa, of which 32 are exotic species (Jordaan, 1993). For comparison, in the British Isles 136 species have been recorded, of which 71 are exotic (Clapham *et al.*, 1962), and in Israel a total of 80 brassica species have been recorded (Zohary, 1966). By contrast, in Taiwan, where DBM is exotic and a severe pest, Liu and Ying (1976) recorded only 19 brassicas, none of which is considered indigenous.

D. collaris is arrhenotokous in South Africa whereas it is thelytokous in Europe (M. Fitton, personal communication; R. Zwart, personal communication), and in Taiwan (W.Y. Su, personal communication). According to Mayr (1965), all asexual organisms seem to be derived from sexual forms. Asexuality in existing organisms is almost certainly a secondary phenomenon (Dougherty, 1955; Stebbins,1960). A hyperparasitoid, *Mesochorus nigripes* Ratzeburg, was accidentally introduced from Sweden into Colorado in the United States when European parasitoids were introduced for biological control of the Alfalfa weevil, *Hypera postica* (Gyllenhal). Only a unisexual form of *M. nigripes* was subsequently observed in Colorado, whereas only a bisexual form exists in Europe (Coseglia *et al.*, 1977). A genetic variability study of these two forms indicated that Europe was the origin of the unisexual form now found in the United States (Hung *et al.*, 1988). Similarly, *D. collaris* may have evolved in South Africa and dispersed to Europe.

The rich fauna of DBM parasitoids, the large number of indigenous brassicas and the arrhenotokous form of the DBM parasitoid, *D. collaris*, are indications that DBM probably evolved in South Africa. As biological control workers have been focusing on Europe as a source of natural enemies against DBM for introductions to other continents, it is suggested that more attention should be given to southern Africa.

Acknowledgements

I thank Sean Moore, Wimpie Roux and Reneè Dehning (Plant Protection Research Institute, Pretoria) for technical assistance. N.J. Van Rensburg and M.W. Mansell (PPRI, Pretoria) for criticism. The parasitoids were identified by G.L. Prinsloo and C.D. Eardley (PPRI, Pretoria), D.A. Barraclough (Natal Museum, Pietermaritzburg), R. Zwart (Wageningen Agricultural University), A.K. Walker and J. LaSalle (International Institute of Entomology, London) and M. Fitton and T. Huddleston (Natural History Museum, London). The pheromone dispensers were supplied by AgriSense-BCS Limited, UK.

References

Alam, M. M. (1992). Diamondback moth and its natural enemies in Jamaica and some other Caribbean islands. In *Management of Diamondback Moth and Other Crucifer Pests: Proceedings of the Second International Workshop*. (ed. N.S. Talekar) pp. 233–243. Asian Vegetable Research and Development Center. Shanhua, Taiwan

Annecke, D. P. and Moran, V. C. (1982). Insects and Mites of Cultivated Plants in South Africa. Butterworths, Durban, South Africa, 383 pp.

Boucek, Z. (1988). Australian Chalcidoidea (Hymenoptera). A biosystematic revision of genera of 14 families, with a reclassification of species. CAB International, Wallingford, UK

Broodryk, S. W. (1969). The biology of *Chelonus (Microchelonus) curvimaculatus* (Cameron) (Hymenoptera: Braconidae). *Journal of the Entomological Society of Southern Africa* 32: 169–189

Clapham, A. R., Tutin, T. G. and Warburg, E. F. (1962). *Flora of the British Isles*. University Press, Cambridge

Cock, M. J. W. (1983). Introduction of parasites of *Plutella xylostella* (L.) into Republic of Cape Verde Islands. Project Report, Commonwealth Institute of Biological control. (unpublished)

Cock, M. J. W. (1985). A review of biological control of pests in the Commonwealth Caribbean and Bermuda up to 1982. Technical Communication 9. Commonwealth Institute of Biological Control, Commonwealth Agricultural Bureaux, 218 pp.

Coseglia, A. F., Simpson, R. G. and Eklund, L. R. (1977). Biology of *Mesochorus nigripes*: a hyperparasite of *Bathyplectes* spp. *Annal of the Entomological Society of America* 70: 695–698

Dennill, G. B. and Pretorius, W. L. (1995). The status of diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae), and its parasitoids on cabbages in South Africa . *African Entomology* 3: 6 5–71.

Dougherty, E. C. (1955). The origin of sexuality. *Systematic Zoology* ***4:*** 145–169.

Fitton, M. (1995). Personal communication. Natural History Museum, London, UK.

Fitton, M. and Walker, A. (1992). Hymenopterous parasitoids associated with diamondback moth: the taxonomic dilemma. In *Management of Diamondback Moth and Other Crucifer Pests: Proceedings of the Second International Workshop*. (ed. N.S. Talekar) pp. 225–232. Asian Vegetable Research and Development Center. Shanhua, Taiwan

Gunn, D. (1917). The small cabbage moth (*Plutella maculipennis* Curtis). *Bulletin, Department of Agriculture, Union of South Africa* ***8:*** 1–10

Harcourt, D. G. (1960). Biology of diamondback moth, *Plutella maculipennis* (Curt.) (Lepidoptera: Plutellidae), in Eastern Ontario III. Natural enemies. *The Canadian Entomologist* ***92:*** 419–428.

Hardy, J. (1938). *Plutella maculipennis* Curt., its natural and biological control in England. *Bulletin of Entomological Research* ***29:*** 343–372

Hemy, C. (1984). Growing Vegetables in South Africa. Macmillan, Johannesburg

Hung, A. C.F ., Day, W. H. and Hedlund, R. C. (1988). Genetic variability in arrhenotokous and thelytokous forms of *Mesochorus nigripes* (Hym.: Ichneumonidae). *Entomophaga* ***33:*** 7–15

Jordaan, M. (1993). Brassicaceae. In *Plants of Southern Africa: Names and Distribution. Memoirs of the Botanical Survey of South Africa* 62 (ed. Arnold, T.H. and De Wet, D.C.) pp. 313–322

Kfir, R. (1990). Parasites of the spotted stalk borer, *Chilo partellus* (Lepidoptera: Pyralidae) in South Africa. *Entomophaga* ***35:*** 403–410

Kfir, R. (1992). Seasonal abundance of the stem borer *Chilo partellus* (Lepidoptera: Pyralidae) and its parasites on summer grain crops. *Journal of Economic Entomology* ***85:*** 518–529

Kfir, R. (1995). Parasitoids of the African stem borer, *Busseola fusca* (Lepidoptera: Noctuidae), in South Africa. *Bulletin of Entomological Research* ***85:*** 369–377

Kfir, R., J. Gouws and S. D. Moore. (1993). Biology of *Tetrastichus howardi* (Olliff) a facultative hyperparasite of stem borers. *Biocontrol Science and Technol ogy* ***3:*** 149–159

Lim, G. S. (1986). Biological control of diamondback moth. In Talekar, N. S., and Griggs, T. D. (ed.) Diamondback Moth Management: Proceedings of the First International Workshop, Asian Vegetable Research and Development Center, Shanhua, Taiwan, 159–171

Lim, G. S. (1992). Integrated pest management of diamondback moth: practical realities. In *Management of Diamondback Moth and Other Crucifer Pests: Proceedings of the Second International Workshop*. (ed. N.S. Talekar) pp. 565–576. Asian Vegetable Research and Development Center. Shanhua, Taiwan

Liu, T. S. and Ying, S. S. (1976). Cruciferae. In *Flora of Taiwan* Vol. II Angiospermae. (ed. H.L. LIN *et al*.) pp. 675–700. Taipei: Epoch Publishing Company

Mayr, E. (1965). Animal Species and Evolution. Belknap Press of Harvard University Press, Cambridge, Mass. and London, England

Muggeridge, J. (1930). The diamondback moth. Its occurrence and control in New Zealand. *New Zealand Journal of Agriculture* ***42:*** 253–264

Moore, S. D. and Kfir, R. 1995a. Host preference of the facultative hyperparasitoid *Tetraschus howardi* (Olliff) (Hymenoptera: Eulophidae). *Entomophaga* ***40:*** 69–76

Moore, S. D and Kfir, R. 1995b. Aspects of the biology of the parasitoid *Tetrastichus howardi* (Olliff) (Hymenoptera: Eulophidae). *Journal of African Zoology* ***109:*** 455–466

Mushtaque, M. (1990). Some studies on *Tetrastichus sokolowskii* Kurd, (Eulophidae: Hymenoptera), a parasitoid of diamondback moth in Pakistan. *Pakistan Journal of Zoology* ***22:*** 37–43

Ooi, P. A. C. (1988). Laboratory studies of *Tetrastichus sokolowskii*. *Entomophaga* ***33:*** 145–152

Ooi, P. A. C. and LIM, G.S. (1989). Introduction of exotic parasitoids to control diamondback moth in Malaysia. *Journal of Plant Protection in the Tropics* ***6:*** 103–111

Poelking, A. (1992). Diamondback moth in the Philippines and its control with *Diadegma semiclausum*. In *Management of Diamondback Moth and Other Crucifer Pests: Proceedings of the Second International Workshop*. (ed. N.S. Talekar) pp. 271–278. Asian Vegetable Research and Development Center. Shanhua, Taiwan

Robertson, P. L. (1939). Diamondback moth investigation in New Zealand. *New Zealand Journal of Science and Technology 20:* 330–364

Stebbins, G. L., Jr. (1960). The comparative evolution of genetic systems. In: S. Tax, ed. The Evolution of Life. University Chicago Press, Chicago, pp. 197–226

Su, W. Y. (1996). Personal communication. Department of Biopesticides, TACTRI, Taichung, Taiwan

Syed, A. R., Abdul Aziz, A. A. M., Loke, W. H., Lim, G. S. and Rani, M. Y. (1990). IPM of DBM: development and evaluation. In *Management of Plutella xylostella in Malaysia: Prospects and Strategy.* Kuala Lumpur: Malaysian Agricultural Research and Development Institute. 39 pp

Talekar, N. S. and Shelton, A. M. (1993). Biology, ecology, and management of the diamondback moth. *Annual Review of Entomology* ***38:*** 275–301

Taylor, J. S. (1932). Report on cotton insect and disease investigations. Part II. Notes on the American bollworm, *Heliothis obsoleta* Fabr, on cotton and its parasite *Microbracon brevicornis* Wes. *Science Bulletin, Department of Agriculture, South Africa* ***113:*** 1–18

Ullyett, G. C. (1947). Mortality factors in populations of *Plutella maculipennis* Curtis (Tinedae: Lep), and their relation to the problem of control. *Entomology Memoirs, Department of Agriculture and Forestry, Union of South Africa* ***2:*** 77–202

Van Den Berg, H., Waage, J. K. and Cock, M. J. W. (1988). Natural enemies of *Helicoverpa armigera* in Africa: a review. 81 pp. London, CAB International Institute of Biological Control

Walker, A. and Fitton, M. G. (1992). Records of recent hymenopterous parasitoids attacking diamondback moth in Africa. *Newsletter. IOBC Global Working Group on Biological Control of* Plutella pp. 8

Wilson, F. (1960). *A review of the biological control of insects and weeds in Australia and Australian New Guinea. Technical Communication No. 1.* Ottawa: Commonwealth Institute of Biological Control. 102 pp

Zohary, M. (1966). *Flora Palaestina.* Israel Academy of Sciences and Humanities, Jerusalem

Zwart, R. (1995). Personal communication. Wageningen Agricultural University, Netherlands

A survey of insect parasitoids of *Plutella xylostella* and the seasonal abundance of the major parasitoids in Hangzhou, China

Liu Shu-sheng[1], Wang Xin-geng[1], Guo Shi-jian[2], He Jun-hua[1] and Song Hui-ming[2]

[1]Department of Plant Protection, Zhejiang Agricultural University, Hangzhou 310029;
[2]Institute of Plant Protection, Zhejiang Academy of Agricultural Sciences, Hangzhou 310004, China

Abstract

We conducted an investigation of insect parasitoids of the diamondback moth, *Plutella xylostella*, in crucifer vegetable crops in the suburbs of Hangzhou in four periods from 1989 to 1996. The following six species of primary parasitoids were recorded: *Trichogramma* sp., *Cotesia plutellae*, *Oomyzus sokolowskii*, *Diadromus collaris*, *Itoplectis naranyae*, *Brachymeria excarinata*. Also recorded were seven species of hyperparasitoids of *C. plutellae*. Rates of parasitization of eggs were usually very low or none. However, rates of parasitization of larvae and pupae usually were substantial and showed two peaks each year, around June-July and September–November, respectively. During the peaks the rates of parasitization were usually in the range of 10–60% and reached over 80% on a few occasions. *C. plutellae*, *O. sokolowskii* and *D. collaris* were the major larval, larval-pupal and pupal parasitoids, respectively. In the field , *C. plutellae* was active throughout the year. *O. sokolowskii* was active from May to October, entered into a quiescent state at the pupal stage in October–November to overwinter and would not emerge until April–May next year. *D. collaris* usually was recorded only from May to July. It was noted that rates of parasitization of the diamondback moth in radish and mustard fields were usually higher than those in common cabbage and Chinese cabbage fields in the same locality. It was also noted that there were negative correlation of the rates of parasitization between the two major larval parasitoids, *C. plutellae* and *O. sokolowskii*, indicating that there may be competitive relationship for host larvae between the two parasitoids.

Key words: *Plutella xylostella*, insect parasitoids, rates of parasitization

Introduction

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), was a minor pest of crucifer vegetable crops in China before the 1960's. Its pest status increased rapidly since early sixties when chemical insecticides started to be widely applied in vegetable crops. In the last twenty years, this insect has been the most important insect pest of crucifer vegetable crops, especially in the Changjiang River Valley and Southern China (Shi and Liu, 1995). It has developed resistance to all groups of insecticides, including insect growth regulators and *Bacillus thuringiensis* Berliner (Bt) (Chao *et al*., 1991; Tang *et al*., 1992). Development and implementation of integrated pest management (IPM) systems are now considered to be the only solution to combat this highly resistant insect pest (Liu *et al.*, 1995).

In the past twenty years, development and implementation of biological control-based IPM has made remarkable achievement in the management of DBM in many parts of the world, such as in Southeast Asia and USA (Talekar and Shelton, 1993; Ooi, 1992; Biever, 1994). In these IPM systems, introduction and augmentation of insect parasitoids have played a key role.

On the mainland of China, preliminary surveys of insect parasitoids of DBM have been conducted in Hubei (Lu, 1983), Guangzhou (Chen *et al*., 1987), Beijing (Wu *et al*., 1987) and Hangzhou (Ke and Fang, 1982). A total of 16 species have been recorded, but the majority of them have not been identified to the species. Little is known about the impact of insect parasitoids on the populations of DBM. As part of an effort to help develop biological control-based IPM of DBM in the Changjiang River Valley, we conducted a survey of insect parasitoids of DBM in Hangzhou suburbs and observed the seasonal changes of rates of parasitization. We also investigated the patterns of seasonal abundance of the major parasitoids.

Materials and Methods

Two types of sampling, i.e., irregular sampling in sprayed plots and regular sampling in unsprayed plots, were carried out in the suburbs of Hangzhou, where a vegetable cropping system typical for the Changjiang River Valley has existed for many years. For the irregular sampling in sprayed plots, sampling was done 1–2 times every month. In each time of sampling, samples were taken from one or more plots of different crops. These plots were not chosen at random. They were chosen on the basis of (1) no spray of chemical insecticides in the past 10 days and (2) relative high number of DBM. Irregular sampling was conducted for four periods of time from 1989 to 1996: September–December 1989, June–July 1990, June–November 1994, and May 1995–April 1996. For the

sampling in unsprayed plots, sampling was made at intervals of 5–10 days from transplanting to harvest. Regular sampling was conducted in three plots in September–October 1989, June–July 1990, and May 1995–January 1996, respectively.

The following sampling methods were used for both types of sampling. On each sampling date, depending on the density of DBM, 10 to 50 plants were chosen at random on each plot of crop (the size of one plot varied in the range of 0.03–0.07 ha). All larvae (with exception of first instar larvae), prepupae, pupae and parasitoid pupae were removed from each plant and taken back to the laboratory. Thirty to 50 DBM eggs were also taken from the plants. In the laboratory, DBM eggs were kept in groups of 5–10 in glass vials. DBM larvae were retained singly on fresh cabbage leaves in glass vials. DBM prepupae and pupae, and parasitoid pupae were kept singly in glass vials. All the vials were kept at room temperatures except in July–August when they were kept at 25 °C in a temperature-controlled room because of the high ambient temperature. DBM eggs were kept until eclosion or parasitoid emergence. DBM larvae, prepupae and pupae, and parasitoid pupae were reared until emergence of parasitoid adults or emergence of DBM moths. Dead individuals were dissected to see whether there were parasitoid larvae or eggs inside.

Results

During the four periods of this study, 83 samples in all were taken from common cabbage, Pak-choi, cauliflower, radish and mustard. A total of 4050 eggs, 10550 larvae and 5060 pupae of DBM were sampled and reared in the laboratory.

Species of parasitoids

Six primary parasitoids were recorded *(Table 1).* Of the 6 species, *Oomyzus sokolowskii* is the only aggregate parasitoid, all the rest 5 species are solitary parasitoids. *Cotesia plutellae* and *O. sokolowskii* were most abundant, and *Diadromus collaris* was also abundant at times (see below). These three species can be regarded as the major species. It was noted that *O. sokolowskii* is also a major hyperparasitoid.

Seven species of parasitoids of *Cotesia plutellae*, i.e., hyperparasitoids of DBM, were recorded as follows: *Eurytoma verticillata* (Fabricius) (Hymenoptera: Eurytomidae), *O. sokolowskii*, *Ceraphron manilae* (Ashmead) (Hymenoptera: Ceraphronidae), *Trichomalopsis apanteloctenus* (Crawford), *T. shirakii* (Crawford), *Trichomalopsis* sp.1 and *Trichomalopsis* sp. 2 (Hymenoptera: Pteromalidae). Of the seven species, *E. verticillata* and the 4 species of *Trichomalopsis* are solitary parasitoids, while *O. sokolowskii* and *C. manilae* are aggregate parasitoids.

Rates of parasitization of DBM eggs

Rates of parasitization of DBM eggs were low throughout the study periods, usually in the range of 0–5%. However, in one plot of radish sampled during September–October, rates of parasitization were substantial, reaching 62.9% at its peak.

Rates of parasitization of DBM larvae

Rates of parasitization varied greatly with time. There were also a lot of variations between plots of the same crop and between plots of different crops at the same time of the year. These variations were not unexpected, especially in view of the wide variations of spray of chemical insecticides in different plots by different farmers. Nevertheless, the data of *Table 2* show that parasitization of larvae was substantial on many occasions despite of the heavy input of chemical insecticides into the crop system. The rates of parasitization were in general highest during June–July and September–November each year, when DBM population was most abundant. During these two peak periods, rates of parasitization were usually in the range of 10–60%. It can be seen from *Table 2* that the parasitization was mainly done by two parasitoids, i.e., *C. plutellae* and *O. sokolowskii*. It was also observed that *C. plutellae* was parasitized by a number of secondary parasitoids, and the rates of parasitization reached over 20% on many occasions.

Simple comparison between crops indicates that on the whole the rates of parasitization in radish and mustard were higher than those in cabbage, Pak-choi and cauliflower. Because the sprays of chemical insecticides and the number of DBM larvae in different plots of crops were not recorded, it is not possible to speculate any particular reasons for the differences.

Field and laboratory observation on *C. plutellae* showed that this parasitoid was active throughout the year, although it had very low numbers and developed slowly in winter months.

Figure 1 shows the results from an unsprayed plot of common cabbage in autumn to winter 1995. DBM larvae increased rapidly initially to 25 per plant, then decreased to 10–15 per plant and maintained at that level to the end of the crop. Parasitoids of larvae were active throughout the crop period. Following the peak of DBM larvae, there was an apparent increase in rates of parasitization to nearly 50%. Decrease of parasitism during the late part of the crop was accompanied by low temperatures apparently unfavorable to the parasitoids during that time of the year.

Detailed examinations of the rates of parasitization by each of the larval parasitoids indicate likely negative correlation of rates of parasitization between the two major larval parasitoids, *C. plutellae* and *O. sokolowskii*. Higher rates of parasitization of one species were usually accompanied by lower rates of the other species *(Table 2).* The phenomenon was also demonstrated by the data from unsprayed plots *(Figure 2).*

Rates of parasitization of DBM pupae

The data in *Table 3* and *Figure 1* show that the patterns of parasitization of pupae, including seasonal variations and variations between plots and crops, were similar to those of larvae described above. Highest

Table 1. Hymenopterous parasitoids of diamondback moth recorded from Hangzhou, China

Species	Stages of DBM attacked	Relative importance[a]
Ichneumomonidae		
Diadromus collaris (Gravenhorst) [b]	Pupa	++
Itoplectis naranyae (Ashmead)[b]	Pupa	+
Braconidae		
Cotesia plutellae Kurdjumov	Larva	+++
Eulophidae		
Oomyzus sokolowskii Kurdjumov	Larva-pupa	+++
Chalcididae		
Brachymeria excarinata Gahan [b]	Pupa	+
Trichogrammatidae		
Trichogramma sp.	egg	+

[a]+++ abundant and most important, ++ frequently seen and important, + occasionally seen
[b]New records from China

Table 2. Rates of parasitization of diamondback moth larvae in sprayed plots in Hangzhou, China

Date (yr./ month)	Crop	No. of samples	Total no. of larvae sampled	% parasitized in each sample	% parasitized by major species		% of *C. plutellae* parasitized[a]
					C. plutellae	*O. sokolowskii*	
89. 9	Cauliflower	2	157	3.9, 13.8	0	3.9, 13.8	0
10	Cabbage	1	130	0			
10	Cauliflower	2	160	46.7, 50.0	0, 31.3	18.8, 46.7	0, 15.6
10	Radish	2	163	17.5, 44.7	0, 4.1	17.5, 40.7	0, 23.5
11	Cauliflower	2	72	0, 33.3	0, 7.4	0, 25.9	0, 9.1
12	Cauliflower	2	240	0			
90. 6	Cabbage	3	120	0			
6	Pak-choi	3	520	0–57.5	0–16.7	0–53.9	0
7	Pak-choi	4	292	18.7–58.3	0–31.0	15.6–50.0	0
94. 6	Pak-choi	5	370	10.2–50.0	10.2–50.0	0	0
7	Pak-choi	2	390	0, 7.7	0	0, 7.7	0
10	Cabbage	2	280	10.0, 46.3	0, 3.6	10.0, 46.3	0
11	Cabbage	2	330	0, 11.6	0	0, 11.6	0
95.5	Cabbage	3	520	0-8.8	0–8.8	0	0
6	Cabbage	5	1 064	2.0–22.7	2.0–22.7	0	0
6	Mustard	2	252	59.7, 84.3	59.7–84.3	0	22.6, 25.0
7	Radish	2	654	49.7, 51.8	49.7, 51.8	0	5.3, 20.5
7	Pak-choi	4	575	0-24.4	0–24.4	0	0, 20.0
8	Radish	1	360	69.4	69.4	0	0
8	Mustard	1	223	21.1	21.1	0	0
8	Pak-choi	2	416	26.7, 58.9	26.7, 58.9	0	0, 20.0
8	Cabbage	4	200	0–40.0	0–40.0	0	0
9	Cabbage	7	716	4.0–22.0	4.0–22.0	0–4.2	0
10	Cabbage	5	418	3.6-42.2	3.6–41.5	3.6–20.0	0
11	Cabbage	4	319	25.5–53.3	25.5–55.3	0–25.0	0
12	Cabbage	3	352	5.0–15.0	5.0–15.0	0	0
96.1	Cabbage	3	405	0–3.0	0-3.0	0	0
2	Cabbage	1	114	0			
3	Cauliflower	1	150	0			
4	Cauliflower	4	239	0–3.5	0–2.0	0–1.3	0

[a] % of *C. plutellae* parasitized by secondary parasitoids.

rates of parasitization also occurred in June-July and October–November each year when DBM was most abundant. The rates during these peak periods were usually in the range of 10–60%, but reached 80–90% on several occasions. The parasitization was done mainly by two species, *O. sokolowskii* and *D. collaris*. *O. sokolowskii* was observed in the field from April to December each year, but *D. collaris* was observed only from May to July.

We observed the distribution and sex ratio of the adults of *O. sokolowskii* coming out from 556 parasitized pupae of DBM. The average number of parasitoids per DBM pupa was 7.8±3.3, ranging from 1 to 23. Females accounted for 85.1% of total number of parasitoid adults. Of the 556 parasitized pupae, 18.4% produced only females, with an average of 6.3 individuals per pupa. These figures compare favorably with those reported by Ooi (1988).

Observations were made on the overwintering of *O. sokolowskii*. Immature individuals collected before early October developed normally to adult emergence. From mid October onwards, an increasing proportion of individuals remained at the prepupal stage and would not develop to adult emergence until April–May

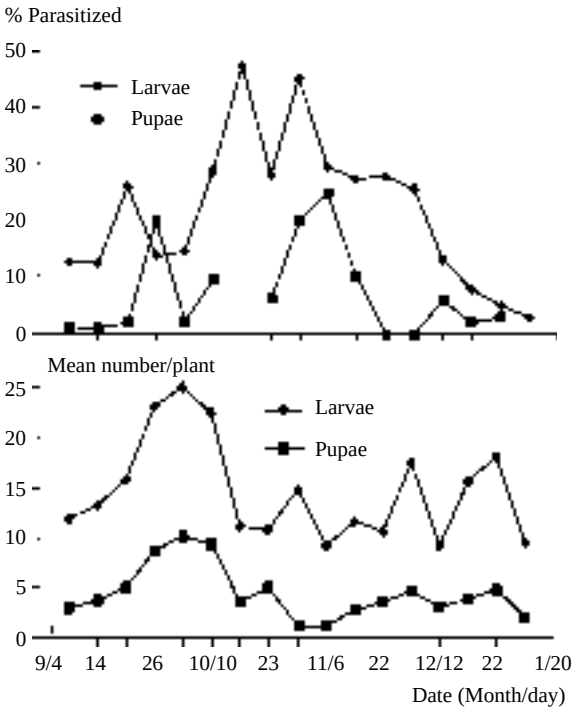


Figure 1. Changes of mean numbers of diamondback moth larvae and pupae, and percentages of diamondback moth larvae and pupae parasitized by insect parasitoids in an unsprayed plot of common cabbage in autumn to winter 1995 in Hangzhou, China

of the coming year *(Table 4). Table 4* also shows that the individuals parasitizing host larvae entered into a state of overwintering earlier than those parasitizing pupae collected on the same day. Because *O. sokolowskii* is a larval-pupal parasitoid, a proportion of individuals collected from host pupae probably started their parasitization earlier when the hosts were at the larval stage, and the parasitoids had already developed to the pupal stage at the time of collection. These parasitoid pupae would keep on normal development to adult emergence.

Discussion

In the suburbs of Hangzhou, DBM is attacked by at least six primary parasitoids, of which *C. plutellae*, *O. sokolowskii* and *D. collaris* are the three major species. In this study, investigations of parasitization of DBM were conducted in both sprayed and unsprayed plots of crucifers. However, strictly speaking, the terms "sprayed" and "unsprayed" are not precise for what they describe. For the feasibility of sampling, the sprayed plots chosen for sampling in general received less chemical insecticides than average and also had higher density of DBM. Meanwhile, the occurrence and development of insect populations in unsprayed plots were undoubtedly affected by the heavy sprays in surrounding plots

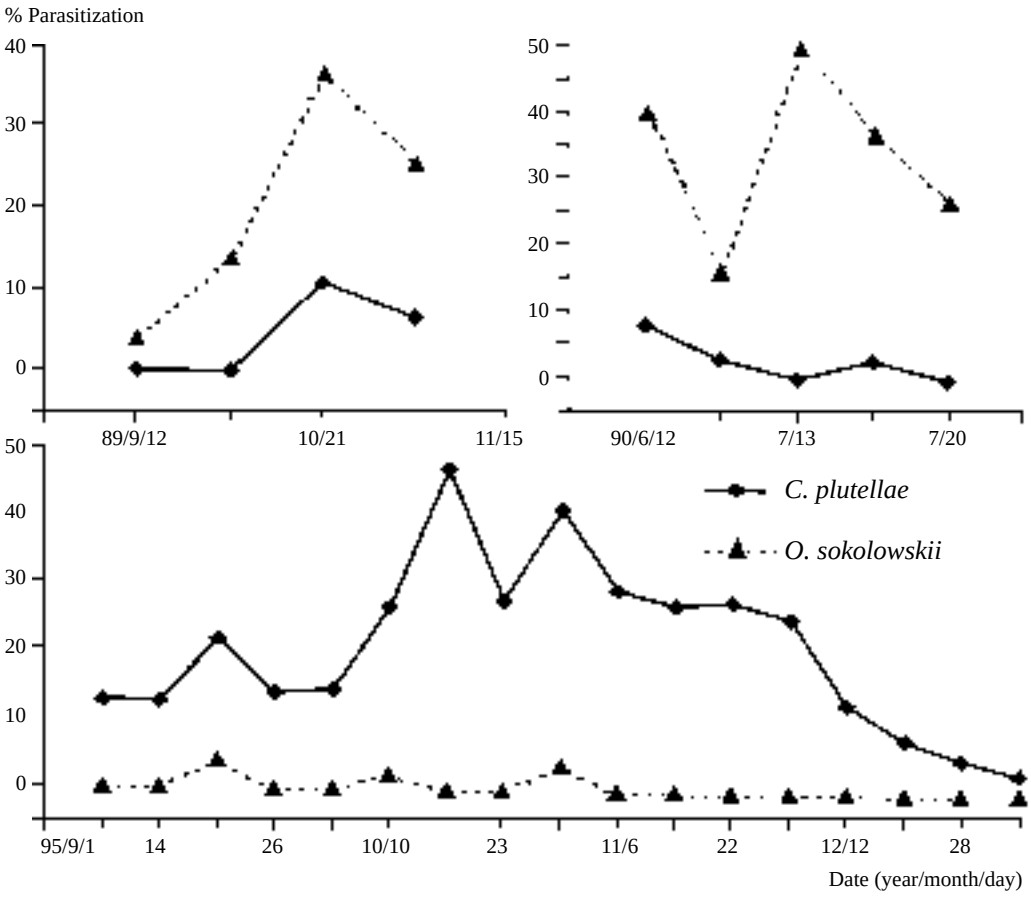


Figure 2. Changes of percentages of diamondback moth larvae parasitized by Cotesia plutellae and Oomyzus sokolowskii in a plot of cauliflower in 1989, in a plot of Pak-choi in 1990, and in a plot of common cabbage in 1995, in Hangzhou, China

Table 3. Rates of parasitization of diamondback moth pupae in sprayed plots in Hangzhou, China

Date (yr. month)	Crop	No. of samples taken	Total no. of pupae sampled	% parasitized in each sample	% parasitized by major species	
					O. sokolowskii	*D. collaris*
89. 9	Cauliflower	2	67	31.3, 50.9	31.3, 50.9	0
9	Cabbage	1	120	0		
10	Pak-choi	1	100	0		
10	Radish	3	148	79.2–91.7	79.2–91.7	0
10	Cauliflower	4	220	28.6–87.5	28.6–87.5	0
10	Cabbage	1	85	5.9	5.9	0
11	Cauliflower	2	88	30.0, 83.8	30.0, 80.8	0
12	Pak-choi	3	156	3.0–39.1	3.0–39.1	0
90.6	Cabbage	3	86	0–20.0	0	0
6	Pak-choi	2	47	8.3, 33.3	8.3, 33.3	0
7	Pak-choi	5	378	41.2–82.5	2.9–82.5	0–48.5
94.6	Pak-choi	4	123	0–21.1	0–18.5	0–21.1
7	Pak-choi	1	70	42.9	42.9	14.3
7	Cabbage	1	100	99.0	99.0	0
10	Cabbage	2	142	15.0, 57.1	15.0, 57.1	0
11	Cabbage	2	131	43.1, 44.0	43.1, 44.0	0
95.5	Cabbage	3	99	0–28.0	0	0–28.0
6	Cabbage	6	965	0–5.8	0	0–5.8
6	Mustard	2	85	35.9, 46.9	0	35.9, 46.9
7	Radish	1	70	42.9	0	42.9
7	Pak-choi	4	360	5.6–30.0	0	5.6–30.0
8	Pak-choi	2	580	0, 0.8	0	0–0.8
8	Cabbage	4	172	0-4.3	0.0–4.3	0
8	Mustard	1	105	3.3	3.3	0
9	Cabbage	6	588	1.0–20.0	1.0–20.0	0
10	Cabbage	4	219	2.1–25.0	2.1–25.0	0
11	Cabbage	4	149	10.0–30.8	10.0–30.8	0
12	Cabbage	3	166	2.0–6.0	2.0–6.0	0
96.1	Cabbage	2	195	0		
2	Cabbage	1	51	0		
3	Cabbage	1	45	0		
4	Cabbage	3	66	0–7.6	0–4.6	0–3.0

Table 4. Dates of adult emergence of *O. sokolowskii* collected from parasitized diamondback moth larvae or pupae in late autumn and early winter from the field in Hangzhou, China

Dates of collection from the field (yr./ month/date)	Individuals parasitizing host larvae		Individuals parasitizing host larvae	
	% emergence during Oct.-Nov. of the same year	% emergence during April-May of the coming year	% emergence during Oct.-Nov. of the same year	% emergence during April-May of the coming year
89/9/20–10/1	100	0.0	100.0	0.0
10/16	42.0	58.0	89.3	10.7
10/21	0	100.0	72.1	27.9
10/25	0	100.0	60.0	40.0
10/27	0	100.0	20.0	80.0
11/7–12/25	0	100.0	0	100.0
95/10/5			100.0	0.0
12/20			0.0	100.0
12/28			0.0	100.0

because of the small sizes of unsprayed plots. Nevertheless, the data collected in this study suggest strongly that insect parasitoids are active in the fields despite of the heavy spray of chemical insecticides in the crop systems over the years, and they can kill a substantial proportion of DBM population especially during periods when the pest is relatively abundant. Further, detailed investigations of DBM-parasitoids interactions, both in the laboratory and in the field, are warranted for quantifying the impact of parasitoids on DBM.

The negative correlation in rates of parasitization of DBM larvae between *C. plutellae* and *O. sokolowskii* observed in this study suggests some sort of competition between the two parasitoids, which also warrants further investigation.

In many parts of the world, *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae)

has made remarkable contribution to the control of DBM (Talekar and Shelton, 1993). This parasitoid seems to be absent from Hangzhou and has not been recorded from the Changjiang River Valley. The high temperatures in summer in the Changjiang River Valley are probably unfavorable to the survival of this parasitoid, because rates of parasitism by this parasitoid were reduced to very low levels at temperatures above 25 °C (Talekar *et al*., 1992). However, in southern Queensland, Australia, where the weather is also characterized by hot summer, *D. semiclausum* survives well and seems to be an important natural enemy of DBM (John Hargreaves, personal communication). Thus, the introduction of *D. semiclausum* into the Changjiang River Valley may be considered.

Acknowledgements

This study forms part of an Australian-China cooperative project "Improvement of Integrated Pest Management of Brassica Vegetable Crops in China and Australia"(PN 9213), funded by the Australian Centre for International Agricultural Research. We also thank the Science and Technology Council of Zhejiang Province for support of the work conducted from 1989 to 1994.

References

Chao Chien-Zhou, Zhu Guo-Ren and Wu Shi-Chang (1991). Development of insecticide resistance and its management in *Plutella xylostella. World Agriculture*, ***9:*** 30–32 (in Chinese)

Chen Long-Ling, Kuang Ming-Zheng, Zhen Zhi-Dong, Cao Yi and Xu Wan-Weng. (1987). A survey of natural enemies of insect pests of vegetable crops in the suburbs of Guangzhou. In*: Integrated Control of Insect Pests and Diseases in Vegetable Crops* (ed. Fan Hui-Zhong *et al.*), pp. 393–399, Guangzhou Science-Technology Press (in Chinese)

Chien Ching-Chin and Chin Shui-Chen. (1985). The hyperparasitoids of *Apanteles plutellae*, a larval parasitoid of diamondback moth in Taiwan. *Journal of Agricultural Research China*, ***34:*** 341–351 (in Chinese, English, summary)

Biever, K. D., Hostetter, D. L. and Kern, J. R. (1994). Evolution and implementation of a biological control-IPM system for crucifers: 24–year case history. *American Entomologist*, ***40:*** 103–108

Liu Shu-Sheng, Brough, E. J., and Norton, G. A. (1995). ACIAR Workshop Report: Integrated Pest Management in Brassica Vegetable Crops. Cooperative Research Centre for Tropical Pest Management, Brisbane, Australia, 69 pp.

Hargreaves, J. (1996). Personal communication. DPI Redlands Research Station, Cleveland, Qld 4163, Australia

Ke Li-Dao and Fang Ju-Lian. (1982). Studies on the biology of the braconid wasp, *Apanteles plutellae* Kurdjumov. *Acta Phytophylacica Sinica*, ***9:*** 27–33 (in Chinese, English summary)

Lu, Yan-Xiong. (1983). A preliminary survey of natural enemies of larvae of *Plutella xylostella*. *Natural Enemies of Insects*, ***5:*** 188–189 (in Chinese)

Ooi, P. A. C. (1988). Laboratory studies of *Tetrastichus sokolowskii*. *Entomophaga*, ***33:*** 145–152

Ooi, P. A. C. (1992). Role of parasitoids in managing diamondback moth in the Cameron Highlands, Malaysia. In: *Diamondback Moth and Other Crucifer Pests: Proceedings of the Second International Workshop* (ed. N. S. Talekar), pp. 255–262, Asian Vegetable Research and Development Centre, Tainan, Taiwan

Shi Zu-Hua and Liu Shu-Sheng. (1995). A review of research and management of diamondback moth in China. In: ACIAR Workshop Report: Integrated Pest Management in Brassica Vegetable Crops (Liu Shu-sheng, E. J. Brough and G.A. Norton), Cooperative Research Centre for Tropical Pest Management, Brisbane, Australia

Talekar, N. S. and Shelton, A. M. (1993). Biology, ecology, and management of the diamondback moth. *Annual Review of Entomology*, ***38:*** 275–301.

Talekar, N. S., Yang, J. C. and Lee, S.T. (1992). Introduction of *Diadegma semiclausum* to control diamondback moth in Taiwan. In: *Diamondback Moth and Other Crucifer Pests: Proceedings of the Second International Workshop* (ed. N. S. Talekar), pp. 263–270, Asian Vegetable Research and Development Centre, Tainan, Taiwan.

Tang Zhen-Hua, Zhou Cheng-Li, Wu Shi-Chang, Zheng Hui-Zhong, Sheng Hui-Liang and GuYan-Zhen. (1992). Insecticide resistance and the effects of synergists in the diamondback moth from Shanghai. *Acta phytophylacica Sinica*, ***19(2):*** 179–185 (in Chinese, English summary).

Wu Ju-Wen, Wang Jin-Rui, Wang Jun, Shi Ba-Dai, Wang Wei, Wang Bao-Zho, Dong Zhen-Hoa, Wei De-Zhong and Huang Qin-Feng. 1987. A list of natural enemies of insect pests of vegetable crops in Beijing. *Acta Agricultural Boriali-Sinica*, ***2:*** 75–96 (in Chinese, English summary)

Experiences with biological control of diamondback moth in the Philippines

Ofelia D. Ventura

Crop Protection Division, Bureau of Plant Industry, 692 San Andres St., Malate, Metro Manila, Philippines

Abstract

The Philippine-German Biological Plant Protection Project, a joint venture of the Bureau of Plant Industry of the Department of Agriculture of the Philippines and the German Agency for Technical Cooperation (GTZ) had evaluated efficient pest management methods against Diamondback moth (DBM), *Plutella xylostella* (Linnaeus) in the Philippines. The establishment of *Diadegma semiclausum* (Hellen) in cabbage plantations achieved economical effective control of DBM providing savings for farmers of up to ₱20,000 per hectare in a cropping season. Large scale reduction of DBM by *Diadegma* has reduced the number of insecticide applications from 18 to 2 sprayings per season lessening contamination of the environment. Augmentative releases of the bio-agent was unnecessary in cabbage fields where parasitoid established and spraying was discontinued. *Diadegma* can be established at altitude 600 m, and year round savings is realized starting at altitude 1 000 m where *Diadegma* is most effective. To compensate or complement *Diadegma,* the use of *Bacillus thuringiensis (B.t).* based insecticides like Xentari® and Agree® is recommended. Other trials with *Cotesia plutellae*, *Trichogramma* species and botanical insecticide gave insignificant control of DBM.

Key words: Biological control, diamondback moth, *Diadegma semiclausum,* cabbage, Philippines

Introduction

Plutella xylostella L. (Lepidoptera: Yponomeutidae) is considered in tropical countries the most destructive and expensive pest in cabbage production. The small lepidopterous insect, commonly called diamondback moth (DBM) is responsible for total loss of a cabbage crop within a few days or weeks in most growing areas if pest control measures are not undertaken. A survey in the vegetable growing areas of Mt. Province and Benguet provinces in Northern Luzon, Philippines from 1989 to 1991 (Ventura *et al*., 1994) found that the pest management cost of DBM in cruciferous crops averaged ₱20,900 (US$836) per hectare per season in 1991, without ensuring successful harvest. An average of 18 insecticide applications per season was observed. The Center for Research and Communication (1990) reported a total of 6,350 hectares of cabbage area in 1987 which was a 19% decrease compared to 7,830 hectares planted in 1980. The reason for the infamous success of the pest can be seen in its explosive multiplication under dry and warm condition and its protective mode of feeding on the underside of the cabbage leaves which makes application of pesticide difficult for farmers. Insecticides found to be highly effective this year can be quite useless one or two years later, due to rapid development of resistance to all commonly used insecticides. Others are already ineffective against this pest in some areas before they can reach the market due to cross resistance.

Task

Based on the following problems of rising pest management costs in cabbage production, the lack of efficient insecticides for DBM control and a clientele of thousands of resource small-scale farmers, the Philippine-German Biological Plant Protection Project, a bilateral technical cooperation of the Bureau of Plant Industry of the Department of Agriculture and the German Agency for Technical Cooperation (GTZ) evaluated efficient pest management methods against DBM in the Philippines. The project pursued biological pest control of DBM in cabbage to provide small-scale vegetable farmers with an alternative strategy to pesticides; one which is environment friendly, safe to human and sustainable.

The project actively involved in the following fields:

Establishment of *Diadegma semiclausum*

One of the successful approaches in the control of DBM was the establishment of a natural enemy of DBM, the larval parasitoid *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae). Releases of *Diadegma* in New Zealand, Indonesia (Sastrosiswojo and Sastrodihardjo, 1986), Malaysia (Ooi, 1992) and Taiwan (Talekar *et. al*., 1992) were reported to have been successfully established in the highlands and effective against DBM.

In 1988, first tests on biological control of DBM using *Diadegma* imported from Asian Vegetable Research and Development Center (AVRDC), Taiwan were conducted in Trinidad Valley, Baguio and Benguet in the Northern part of Luzon (Pölking, 1992). Later in 1990, *Diadegma* cocoons were brought from Indonesia and a simple mass rearing technique was introduced by Sastrosiswojo (1990). Netted cages (50 x 50 x 50cm) were used for rearing DBM and *Diadegma.* However, using small cages for *Diadegma*

production needed a lot of space, was laborious and quite costly. To avoid constraints mentioned above, an efficient mass rearing system was developed for the parasitoid in the Central Biolab in Manila (König, *et al*., 1993).

Rearing Procedure

The rearing process involves 3 steps: raising of cabbage plants, mass rearing of DBM and mass production of *Diadegma*. The order of sequence is important in order not to result in a breakdown of rearing.

1. **Raising of cabbage plants.** Sow 200 seeds every week and raise cabbage plants to eight weeks. To have an output of 2 000 *Diadegma* cocoons per week, it is necessary to introduce 80–100 plants per week to DBM oviposition cages.
2. **Mass rearing of DBM.** Oviposition is done daily on six (8-week old) cabbage plants inside the oviposition cage. After 24 hours the plants are replaced with new ones and the plants with DBM eggs are kept for the further development of DBM. When DBM has reached the L2-larval stage, one third (1/3) of the plants are kept in a netted cage secured against *Diadegma* for continuous DBM production.
3. **Mass production of *Diadegma.*** The rest of the plants with DBM in L2 stage are transferred into a screenhouse (10 x 2 x 2.5m) for parasitization. The screenhouse is divided into two compartments: one large (8 x 2 x 2.5m) and one small (2 x 2 x 2.5m), both equipped with benches to hold the plants.

Thirty (30) *Diadegma* adults are released for oviposition to the smaller compartment at a ratio of 2 female:1 male once in 1–2 weeks depending on their lifespan. The plants with DBM larvae are kept for two days to allow 80% parasitization of DBM. Later, the plants with parasitized DBM larvae are transferred into the larger compartment for 2–3 weeks for *Diadegma* cocoon development.

The utilization of a screenhouse as parasitization unit has the advantage to avoid overcrowding of the wasps and the sex ratio is kept at approximately 1:1. In addition, the installation is cheaper than working with small cages in the laboratory. The environment simulated is closer to field condition and therefore allows adaptation of the beneficial to the temperature in the field. The set-up is ideal for Manila, especially, where temperatures rise resulting from power interruption during the dry months of the year.

In 1990–1992, the first mass rearing for field release based on the described method was started in Baguio. Releases of adult *Diadegma* wasps took place in Mt. Province and Benguet. Establishment was achieved all along the mountain trail, the main cabbage production area of the Philippines. Surveys in 1992 and 1993 by Amend *et al*. (1994) found the presence of *Diadegma* well established and widespread and noted a drastic decline of DBM populations. This occurrence of *Diadegma* proved the ability to dispersal and to survive under adverse conditions and in times where there are low pest population. The reduced pest status meant that farmers could reduce insecticide applications from the original 18 per season to 1–2 at present which are more targeted to other pests than to DBM. The savings for farmers are up to ₱20.000 per hectare per season.

In 1993, Canlaon City, Negros Oriental, the most important vegetable production area in the Visayas was selected to extend the benefits of *Diadegma* to other cabbage production areas in the Philippines. Canlaon is situated on the slopes of the volcano Mt.Canlaon at an elevation of approximately 600m where crucifers and other highland vegetables are cultivated in an area of about 1 000 hectares extending from an altitude of 600 to 1,100m. In a survey made by Wyrwal (1993), he reported that cabbage farmers sprayed insecticides at an average of 1.7 applications/week (every four days).

Releases were done with adult *Diadegma* wasps (200–300 females:100–150 males per field) and released 4–5 times at weekly intervals beginning 2–4 weeks after transplanting. The fields were monitored two weeks after the first release and stopped in case of establishment. Selected fields for release sites had all-year-round planting of cabbage in close proximity and the farmer cooperators were advised to apply insecticides based on *Bacillus thuringiensis (B.t.)*. Results of the monitoring showed that parasitization of DBM reached 90% in fields with *Diadegma* releases and 60% in fields without actual releases (Felkl *et al.*, 1994).

Due to the success of the establishment in the Mt. Province, Benguet and Canlaon, other rearing stations were established in Central Visayas (Cebu and Mandaue), Western Mindanao (La Paz and Sergio Osmeña), Northern Mindanao (Malaybalay) and Southern Mindanao (Davao). The results in all release areas with altitude >600m were the same: fast establishment of *Diadegma* populations, high parasitization rates (>90%), a wide scale reduction of DBM populations and increase in hectarage of cabbage production.

Mass releases of *Cotesia plutellae*

Diadegma showed to be ineffective in lowlands or under extreme high temperature conditions. Therefore, the indigenous DBM parasitoid *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae) also known as *Apanteles plutellae* was utilized by rearing it following the *Diadegma* mass rearing procedure and released in fields with an altitude up to 400m. Result showed parasitization rates of up to 50% but no real economic reduction of damage was realized (Salazar and Rivera, 1994).

Release of *Trichogramma* species

To deal with DBM at egg stage, and especially to manage other lepidopterous pests without insecticides, *Trichogramma* species *(T. chilonis, T. ostriniae,* and *T. bactrae*) were tested. The results obtained were not

promising. Natural parasitization of DBM eggs observed was up to 87%. Although with the high parasitization, damage of the plants could not be lowered. Therefore, the trials were discontinued.

Use of botanical

A farmer-made insecticide using local plant material (seeds and leaves of madre de cacao, *Gliricidia sepium*) which helped farmers to save on insecticide costs was tested to verify the effect on oviposition behavior of DBM (Stoll *et al*., 1994). The results showed promise, especially for ethanol extracts which reduced DBM oviposition by 70%. Nevertheless, the botanical was not recommended to farmers due to lack of knowledge about possible dangerous side effects of the insecticidal solution and doubts about the acceptance by farmers.

Integrated Pest Management

Diadegma has its limitations. Despite a successful rearing under the extreme climate in Manila, *Diadegma* in the field will reduce parasitization and multiplication during times of high temperature. This is especially true for altitudes below 600 m but the effect of hot temperature can be found up to an altitude of 1 000 m. To cope with this problem it is necessary to resort under certain circumstances to chemical pest control. In order to determine when will farmers resort to pesticides use and which insecticides are compatible to the biological control of DBM by the larval parasitoid *D. semiclausum*, a series of field trials on the control of DBM with microbial and synthetic insecticides and their side effects on *Diadegma semiclausum* was conducted in Canlaon, Negros Oriental (König *et al*., 1995). The results showed that the integration of microbial insecticides, Xentari® (*B.t.* var. *aizawai*) and Agree® (*B.t.* var. *kurstak*i x *aizawai*) among other microbial insecticides achieved the best result in the biological control of DBM while the synthetic insecticides, profenofos and imidachloprid were effective against aphids. As a result, a "Biocontrol against DBM" manual was produced for technicians (König *et al*., 1996) with the following recommendations:

- *Seedbed* : One treatment against aphids with profenofos or imidachloprid
- *Field* : One or two treatments against aphids with profenofos or imidachloprid within the first 3 weeks after planting.
- Monitor weekly and apply bioinsecticide on the whole field if more than 4 DBM larvae per plant are observed. The same applies if more than 20% of the cabbage plants are infested with other pests, especially with cabbagehead caterpillar (*Crocidolomia binotalis* Zell. (Lepidoptera: Pyralidae) or cabbage webworm (*Hellula undalis* F. (Lepidoptera: Pyralidae).
- If only single plants are attacked, spot treatment of the infested plants as well as the neighboring plants is necessary.
- Observe the field in case of periods with unusually high temperatures (>35 °C), and employ preventive measure against DBM. This must be supported by pest management using bioinsecticides.

At present, utilization of *Diadegma* is supported and promoted by the Department of Agriculture through the National Integrated Pest Management – KASAKALIKASAN program. Trained farmers were able to reduce insecticide applications from 18 to 1–2 per season. In most cases sprayings were necessary only to manage other pests: e.g. cabbage cutworm (*Spodoptera litura* F., Lepidoptera: Noctuidae), cabbage looper *(Trichoplusia ni* Hub., Lepidoptera: Noctuidae), cabbage butterfly (*Pieris canidia*, Lepidoptera: Pieridae), and aphids (*Brevicoryne brassicae* L ., Homoptera: Aphididae). The savings are in the range of ₱18 000 to 20 000 per hectare in a season.

References

Amend, J., Mangali, T., and Emaguin, D.P. (1994). Establishment of *Diadegma semiclausum* (Hellen) (Hymenoptera: Ichneumonidae) in the Philippine Cordillera: Status in 1992 and 1993. *The Philippine Journal of Plant Industry* ***59(1–2):*** 103–108

Anonymous. (1990). Agribusiness Factbook and Directory. Center for Research and Communication. Manila, Philippines. 641 pp.

Felkl, G., Gohlicke, H., Bojos, C., and König, K. (1994). Introduction of *Diadegma semiclausum* for the control of diamondback moth in Negros Oriental. *The Philippine Journal of Plant Industry* ***59(4):*** 21–35

König, K., Bojos, C., Felkl, G., and Gohlicke, H. (1995). Control of diamondback moth with microbial and synthetic insecticides and its side effects on *Diadegma semiclausum*. Report to the Philippine-German Biological Plant Protection Project, Bureau of Plant Industry, Manila, Philippines. 11 pp.

König, K., Bojos, C., Gohlicke, H., and Ventura, O.D. (1996). Biocontrol against Diamondback Moth Manual for Technicians. Philippine-German Biological Plant Protection Project, Bureau of Plant Industry, Manila, Philippines. 18 pp.

König, K., Deogracias, L., and Amend, J. (1993). Mass-Rearing and Utilization of *Diadegma semiclausum* Manual. Philippine-German Biological Plant Protection Project, Bureau of Plant Industry, Manila, Philippines. 18 pp.

Ooi, P.A.C. (1992). Role of parasitoids in managing diamondback moth in the Cameron Highlands, Malaysia. *In* Talekar, N.S.(ed.) Diamondback Moth and Other Crucifer Pests: Proceedings of the Second International Workshop, Asian Vegetable Research and Development Center, Tainan, Taiwan 225–262

Pölking, A. (1992). Diamondback moth in the Philippines and its control with *Diadegma semiclausum*. *In* Talekar, N.S.(ed.) Diamondback Moth and Other Crucifer Pests: Proceedings of the Second International Workshop, Asian Vegetable Research and Development Center, Tainan, Taiwan 271–278

Salazar, P., and Rivera, N. (1994). A study on the biology of *Apanteles plutellae* (Kurdj.) and testing on its effectivity. Paper presented during the in-house review, Bureau of Plant Industry, Manila, 16 pp.

Sastrosiswojo, S. (1990). Mass rearing of *Diadegma eucerophaga* and *Apanteles plutellae*, and development of the biological and integrated control of the diamondback moth on cruciferous crops in the Philippines. Report to the Philippine-German Biological Plant Protection Project, Bureau of Plant Industry, Manila, Philippines. 17 pp.

Sastrosiswojo, S., and Sastrodihardjo, S. (1986). Status of biological control of diamondback moth by introduction of parasitoid *Diadegma eucerophaga* in Indonesia. *In* Talekar, N.S., and Griggs, T.D. (eds.) Diamondback Moth Management: Proceedings of the First International Workshop, Asian Vegetable Research and Development Center, Tainan, Taiwan 184–194

Stoll, G., Bojos, C., Schüli, and Klingauf, F. (1994). *Gliricidia sepium* as a possible source of semiochemicals deterring oviposition of *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae). *The Philippine Journal of Plant Industry* ***59(1–2):*** 109–118

Talekar, N.S., Yang, J.C., and Lee, S.T. (1992). Introduction of *Diadegma semiclausum* to control diamondback moth in Taiwan. *In* Talekar, N. S. (ed.) Diamondback Moth and Other Crucifer Pests: Proceedings of the Second International Workshop, Asian Vegetable Research and Development Center, Tainan, Taiwan, 263–270

Ventura, O.D., Fernandez, G.G., and Depositario, D.P.T. (1994). Cropping system monitoring of vegetable farms in Benguet and Mt. Province (1989–91 dry & wet season) Vol. 1–2 *The Philippine Journal of Plant Industry* ***59(1–2):*** 39–102

Wyrwal, G. (1993). Socio-economic key parameters in vegetable cultivation in selected areas of Negros Oriental, Philippines. Report to the Philippine-German Biological Plant Protection Project, Bureau of Plant Industry, Manila, Philippines. 117 pp.

Classical biological control of diamondback moth: the Malaysian experience

Syed, A.R.[1], A. Sivapragasam[2], W.H. Loke[2] & I. Fauziah[2]

[1]MARDI Cameron Highlands, 39007 Tanah Rata Cameron Highlands, Pahang

[2] Strategic, Environment and Natural Resources Center, MARDI,G.P.O. Box 12301, 50774 Kuala Lumpur

Abstract

The diamondback moth (DBM), *Plutella xylostella* (L), has been recorded in Malaysia since 1925. From 1941, it has been the major pest of crucifers grown in the Cameron Highlands and in the lowlands. The insect occurs in high numbers in the drier periods of the year and control has been mainly with the use of insecticides. The indiscriminate use of insecticides on DBM has led to the development of resistance to almost all classes of pesticides including organophosphates, carbamate, pyrethroids, acylureas and *Bacillus thuringiensis* (especially subsp. *kurstaki*).

In 1975, a biological control program for DBM was initiated. In the same year, a braconid parasitoid *Cotesia plutellae* (Kurdj.) was recovered in Cameron Highlands. Parasitism levels ranged from 12 to 36% which was not effective in managing populations of DBM. Between 1975 and 1978, four species of parasitoids were introduced into the Cameron Highlands from India, New Zealand, Australia and Indonesia. Of these, only two became established, namely, *Diadegma semiclausum* Hellen and *Diadromus collaris G*ravenhorst. Within two years, both these parasitoids were recovered in crucifer-cultivated areas in the Cameron Highlands, even though parasitization levels were low. For almost a decade after their release the importance of the parasitoids was not realized and many considered the role of the parasitoids as not important in the management of DBM. In 1987 and 1988, serious outbreaks of DBM occurred in the highlands and lowland areas and which led to the extensive use of pesticides. This resulted in high pesticide residue in leafy crucifers and rejection of most harvested crops by the consumers.

Between 1985 and 1989, studies were conducted to develop an Integrated Pest Management (IPM) package for DBM both for the highlands and lowlands areas of Malaysia. The package using DBM larval threshold levels and level of parasitization as decision tools was succesfully introduced to the crucifer growers in 1989. Also, biological pesticides *i.e. Bacillus thuringiensis* were recommended when populations exceeded the economic threshold levels. Between 1989 to 1993, DBM numbers fluctuated between 0.5 to 1 larva per plant with parasitization levels of 30 to 60% in the crucifer growing areas in Cameron Highlands. The farmers also applied insecticides mainly, *Bacillus thuringiensis* at only 1 to 2 applications per crop cycle compared to 7 to 10 applications before the introduction of IPM package.

Several factors contributed to the success of the biological control programme to manage DBM in Malaysia. These factors include (1) Establishment of mass rearing facilities for parasitoids of DBM and periodic field releases (2) Training programmes intitiated for extension workers and farmers to recognise natural enemies and understand biological control concepts (3) Constant monitoring of insecticide resistance in problem areas where outbreaks occurred (4) Training on pesticide application technology for growers, and (6) Commitment of the government to reduce pesticide usage and use biological control as the core approach in the IPM programme for vegetables in Malaysia.

Key words: Diamondback moth, biological control, experiences, Malaysia

Introduction

The Cameron Highlands is Malaysia's oldest and most important region of vegetable production with about 1,125 ha. currently under cultivation (Hanada, 1987; Taylor *et al.,* 1992). It consists of mountain range with diversely configured valleys of varying width and steepness. It terms of elevation, Cameron Highlands can be viewed to be made up of three zones: Central which is highest (1400–1500 meters above mean sea level) and Northern and Southern Zones, whose elevation are approximately 300–500 meters less than that of the Central Zone (Lim, 1970; Taylor *et. al.,* 1992; Arthurs, 1996). The Southern Zone consists of areas around Ringlet, Boh Road and Bertam Valley. The Central Zone includes areas around Tanah Rata, Brinchang, Mensum Valley, Kea Farm and Sungai Palas while the Northern Zone comprises areas in Kuala Terla, Kampong Raja and Blue Valley.

Cameron Highlands has a cool subtropical type climate with abundance of rainfall. The higher elevation areas (Central Zone) are much cooler (mean daily minimum = 14 ^{o}C, mean daily maximum = 23 ^{o}C) while in the Southern and Northern Zones the temperatures are relatively higher (mean daily minimum = 16 ^{o}C, mean daily maximum = 26 ^{o}C). Rainfall is well distributed in the highland areas and highly variant both within and between different months of the year, ranging from 60 mm to 500 mm

per month and averaging about 2650 mm per year. However, the wet months are generally April, May, October, November and December, while the other months are relatively drier.

There are about 50 types of vegetables cultivated in Cameron Highlands, the most important being the leafy brassicas like cabbage (*Brassica oleracea* L. v*ar capitata*), Chinese cabbage (*B. pekinensis* (Lour.) Rupr.) and Chinese mustard (*B. chinensis* L. v*ar oleifera)*. Most of the vegetables are generally marketed within the country with 20% exported to Singapore daily (Hanada, 1987).

The development of the vegetable industry has been hampered by a number of constraints (Syed and Loke, 1995) and the main constraint being pests and diseases. Ding *et.al.,* (1981) indicated that at least 85% of the vegetable growers reported pests and diseases as the major constraint and 65% used pesticides to overcome the problem. The major problem in the cultivation of cabbage and other crucifers in Cameron Highlands is the Diamondback moth (DBM), *Plutella xylostella* (L.)(Lepidoptera: Yponomeutidae)(Lim, 1982). The DBM was first recorded in Malaysia in 1925 and in Cameron Highlands in 1934, soon after the cultivation of temperate vegetables began (Ooi, 1986). By 1941, it was considered an important pest of crucifers in Malaysia (Corbett and Pagden, 1941).

Major interest and research on DBM in Malaysia began in the early 1960's as a result of heavy crop losses due to the pest. Outbreaks and incidences were reported yearly with resurgences occurring every 2–3 years (Lim, 1974; Sudderuddin and Kok, 1978). Since the 1940's, the main method of control practised by the growers has been the use of synthetic insecticides. Due to the overdependence on insecticides, several pesticide-related problems such as insecticide resistance development, pest resurgence, pesticide residues on the crop and environmental pollution have surfaced and become serious (Lim *et. al.,* 1988).

In the 1970's, an ecological approach was adopted to manage the DBM problem (Ooi and Lim, 1983) Research was intensified in the area of biology, ecology and control tactics, in an attempt to use biological control as the core in an Integrated Pest Management (IPM) programme for DBM. This paper will highlight some of the findings towards that direction giving focus on the success and the problems associated with it.

Biological control of DBM

Before the 1970's, little was known about the ecology of DBM in Malaysia. The only control measure available to the farmers was insecticides. A biological control approach was simultaneously initiated by MARDI (Malaysian Agricultural Research and Development Institute) and the Crop Protection Division of the Department of Agriculture, Malaysia.

Studies by Lim and Ko (1975) resulted in the discovery of *Cotesia plutellae* (Kurdj.)(Hymenoptera: Braconidae) in the Cameron Highlands. This braconid parasitoid probably arrived with cabbage imported from the neighbouring countries. The discovery of *C. plutellae* paved the way for the acceptance of biological control agents as a possible alternative to break the existing pesticide dependency. Although *C. plutellae* was initially recovered in Tanah Rata (Central Zone), subsequent studies showed it to be common and widespread in the country (Lim and Ko, 1975; Ooi and Sudderuddin, 1978; Lim, 1982). Over the various valleys surveyed in Cameron Highlands, Kuala Terla (Northern Zone) had the highest level of parasitization of 48.6%, while Tanah Rata had the lowest level of 12.7%. Parasitization levels in the other valleys were Kampong Raja (Northern Zone) 36.5%, Tringkap (Central Zone) 34.0%, Mensum Valley (Central Zone) 18.1% and Bertam Valley (Southern Zone) 30.1%. (Ooi, 1979). In general, it was encouraging to see high levels of parasitization in areas where high levels of pesticides were used. Although this suggested possible development of field tolerance to pesticides, subsequent studies could not confirm it (Lim, 1982). Between 1975 and 1980 studies were done on *C. plutellae* encompassing various aspects on its biology and ecology (Ooi, 1979; Lim, 1982) and its impact on DBM (Lim *et.al*., 1986). According to Ooi (1979) and Lim *et.al.* (1982), levels of parasitization of DBM by *C. plutellae* was low and insufficient to manage DBM populations in the Cameron Highlands. It was suggested that the IPM programme would be better if there were more species of parasitoids acting on DBM at its different immature stages.

Biological control as core component of IPM

Attempts were made to introduce exotic parasitoids to complement *C. plutellae* (Ooi and Lim, 1983). Altogether 4 parasitoid species were brought in from Australia, India, Indonesia and New Zealand *i.e. Diadegma semiclausum* Hellen (Hymenoptera : Ichneumonidae), *Diadromus collaris* Graven. (Hymenoptera : Ichneumonidae), *Macromalon orientale* Kerrich. (Hymenoptera : Ichneumonidae) and *Tetrastichus sokolowskii* Kurdj. (Hymenoptera : Eulophidae). Of these, only *D. semiclausum* and *D. collaris* became established in Cameron Highlands. *M. orientale* was never released as it failed to breed in the laboratory. In total, the number of adults of *D. semiclausum, D. collaris* and *T. sokolowskii* released at MARDI Research Station Tanah Rata between 1976–1978 were 1202, 1982 and 21, 225, respectively (Ooi, 1979; Ooi and Lim, 1983).

Following the release of these parasitoids in the Cameron Highlands, for more than a decade, the impact of the two parasitoids, *D. semiclausum* and *D. collaris* was not realised and in the minds of many, the introduction was unsuccessful (Ooi, 1992). However, low numbers of *D. semiclausum* and *D. collaris* were recorded between 1980 to 1985 in unsprayed plots at MARDI Research Station Tanah Rata where the introductions were made. Levels of parasitization never exceeded 5% as compared to 30–60% for *C. plutellae* (Syed, unpublished). A general

survey conducted at that time showed that a few samples of DBM collected were parasitised with *D. semiclausum* and *D. collaris* in Tanah Rata, Brinchang, Kea Farm and Mensum Valley, all in the Central Zone ranging from 5 to 10 kilometers from the introduction area. The low numbers of the introduced parasitoids were probably due to the extensive use of pesticides especially the newly introduced acylurea insect growth regulators (IGRs).

In 1987 and 1988 serious outbreaks of DBM occurred in Cameron Highlands due to the development of resistance to the commonly used IGR insecticides (teflubenzuron, chlorfluazuron and diflubenzuron), an organophosphate (methamidophos) and a synthetic pyrethroid (cypermethrin). The resistance ratio (RR) ranged from 10 to 3000 in some populations (Syed, 1992). This encouraged MARDI to formulate and introduce an IPM package to the growers in 1989 (Loke *et.al.*,1992a). The farmers adopted the IPM package to manage DBM as most of their vegetables had high levels of pesticide residues and were rejected by the consumers in Malaysia and Singapore (Ooi, 1992).

The IPM package introduced in Cameron Highlands is based on tentative Economic Threshold Levels (ETL) of DBM larvae on the plants and level of parasitization by the three parasitoids (*C. plutellae, D. semiclausum* and *D. collaris*). Essentially, the role of the parasitoids formed the core to the IPM programme. The IPM approach consisted essentially of a three-tiered ETL which takes into account the percentage parasitization of the DBM larvae. No pesticides were applied when DBM population were less than 4 larvae per plant or less than 7 larvae per plant with parasitization levels equal or more than 40%. For crops with less than 40% parasitization levels and ETL of 4 to 7, only *Bacillus thuringiensis* (Bt) was applied (Loke *et. al*., 1992a).

Success of biological control and IPM

With the success of using the parasitoids, the IPM package was tested at the farm level. Results showed clearly the superiority of the IPM approach over prophylactic control practised by the cabbage growers. Marketable yields were 5–6% higher and up to 6-fold increase in profits were obtained in the IPM plots. The number of insecticide applications were reduced from 7 to 9 times to a maximum of only 3 applications in the IPM plots (Syed *et. al.,* 1992). Furthermore, no insecticide residues were detected in the cabbage harvested from the IPM plots (Loke *et. al.,* 1992a). To implement the IPM package between 1989 and 1990, several training courses for extension agents, seminars and dialogue sessions with farmers and field demonstrations were held in Cameron Highlands. A survey in 1990 (Loke *et. al.,* 1992a) revealed that 54% of the cabbage growers were practising IPM and 86% were keen to implement the programme. Taylor *et. al.* (1992) also found that 74% of the cabbage farmers recognised the DBM parasitoids and 40% use ETL as indicators for pesticide applications.

Ooi (1992) compared DBM populations from unsprayed plots in 1977 and 1989 and showed that populations of DBM in 1989 decreased by about 8 times or more compared with that in 1977. This suggested that the overall DBM problem was very much reduced following the change in pest management practices. The extensive use of Bt (Ooi 1992; Loke *et. al,*1992a; Loke *et.al.,* 1992b) to replace chemical insecticides led to less adverse effect on the parasitoids resulting in reduced populations of DBM. The differences in population of DBM during the 1976–1978 period and 1988–1990 and 1991–1993 periods became more apparent and this was attributed to the impact of the introduced parasitoids, particularly *D. semiclausum* (Ooi, 1992) *(Figure 1)*.

The impact of biological control and IPM can be seen in the DBM populations and parasitization levels in the three Cameron Highlands zones as shown in *Figures 2, 3* and *4*. In this study three farms were selected from each zone and 20 cabbage sampled for DBM and parasitoids every 2 weeks. All larvae and pupae were reared in the laboratory to observe parasitoids emergence. Populations of DBM were low comparatively from 1991 to 1993 in all zones, and parasitization levels were up to 90% in some cases. A survey was also conducted on DBM populations in the different zones in 1990 and 1995 (*Tables 1* and *2*). In 1990, the DBM population ranged from 0.7 to 1.1 per plant which is way below the economic threshold levels. The major parasitoid in the Southern and Northern Zones was *C. plutellae.* In the Central Zone it was *D. semiclausum.* This is probably due to the warmer temperatures in the Southern and Northern Zones. *D. semiclausum* is more adapted to cooler conditions (Arthurs, 1996). On the contrary *C. plutellae* prefers warmer areas and also occurs in the lowland areas of Malaysia (Lim, 1982).

Problems to sustain biological control and IPM

As indicated earlier, with the implementation of IPM, the parasitoids maintained populations of DBM at low levels. However, in the last few years, DBM populations increased significantly in all the zones in Cameron Highlands (*Figures 2, 3,* & *4*). In the Southern and Northern Zones, this trend began in 1994 and became serious in 1996. In the Central zone, increase in DBM populations was observed in the later part of 1995 and in 1996. The survey in 1995 *(Table 2)* showed DBM population ranging from 1.6 to 2.9 per plant and level of parasitization declined as compared to the 1990 *(Table 1)*. Several factors may have contributed to the upsurge in the DBM populations causing periodic outbreaks and crop losses.

First was the development of insecticide resistance in DBM to recommended insecticides. Earlier studies have shown resistance to practically all insecticides used against DBM in Cameron Highlands (Sudderuddin and Kok, 1978; Syed, 1992). Studies in Cameron Highlands (Verkerk *et.al.,* 1996) showed DBM resistance to *B. thuringiensis* subsp. *kurstaki,*

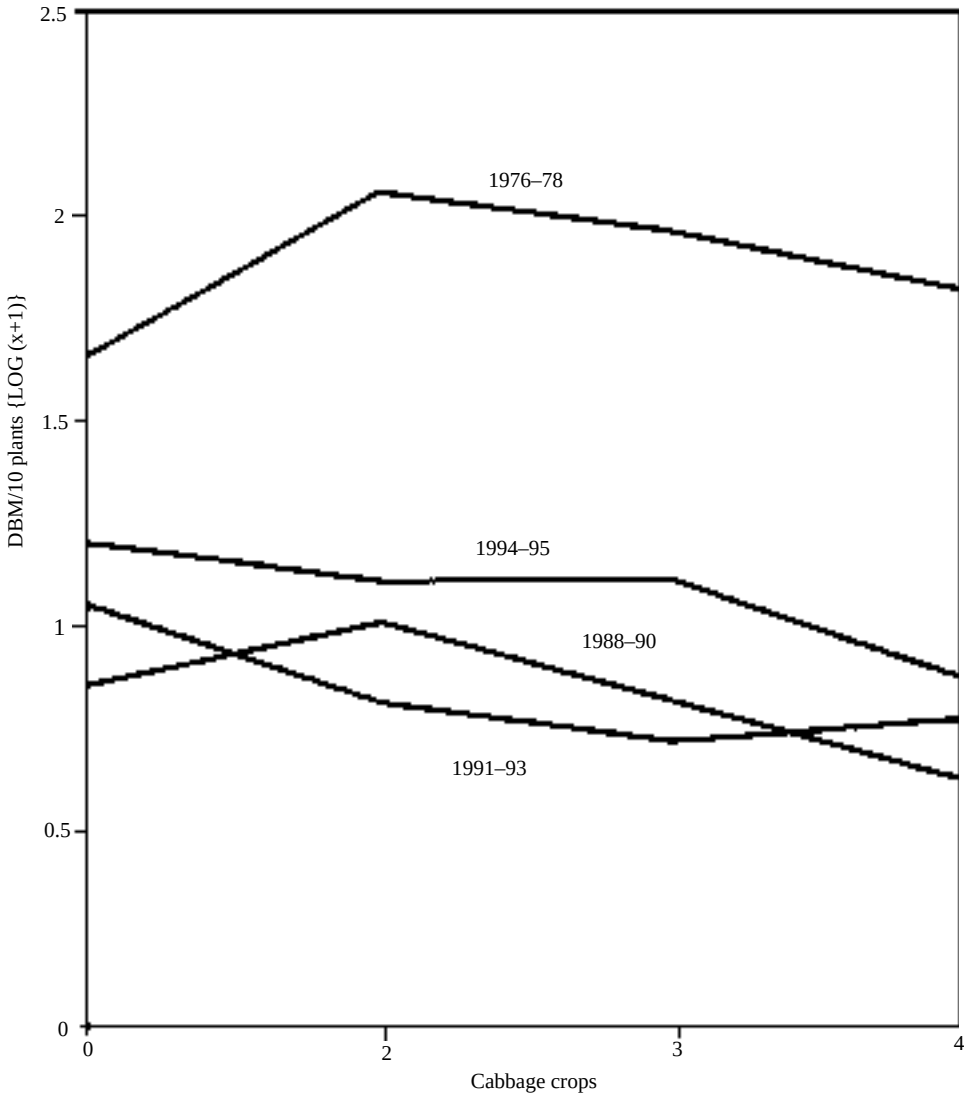


Figure 1. Seasonal population changes of DBM on cabbage in Cameron Highlands in unsprayed plots at MARDI Tanah Rata (modified from Ooi, 1992)

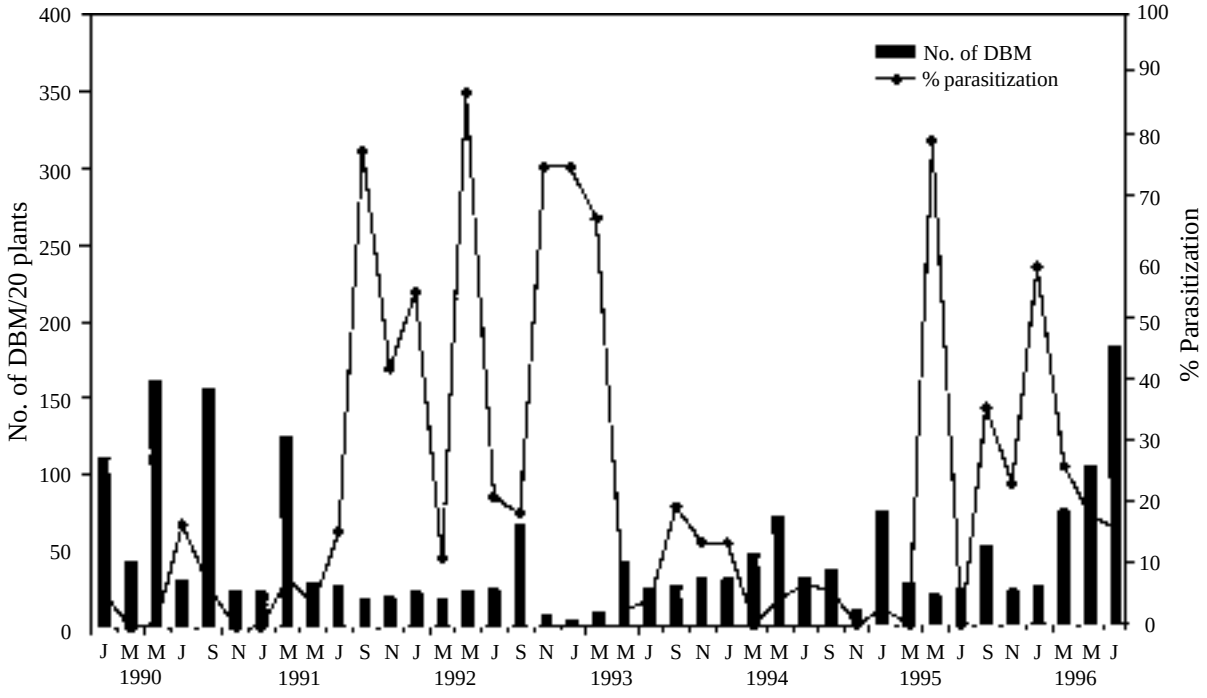


Figure 2. Population of DBM and % Parasitization (Southern Zone)

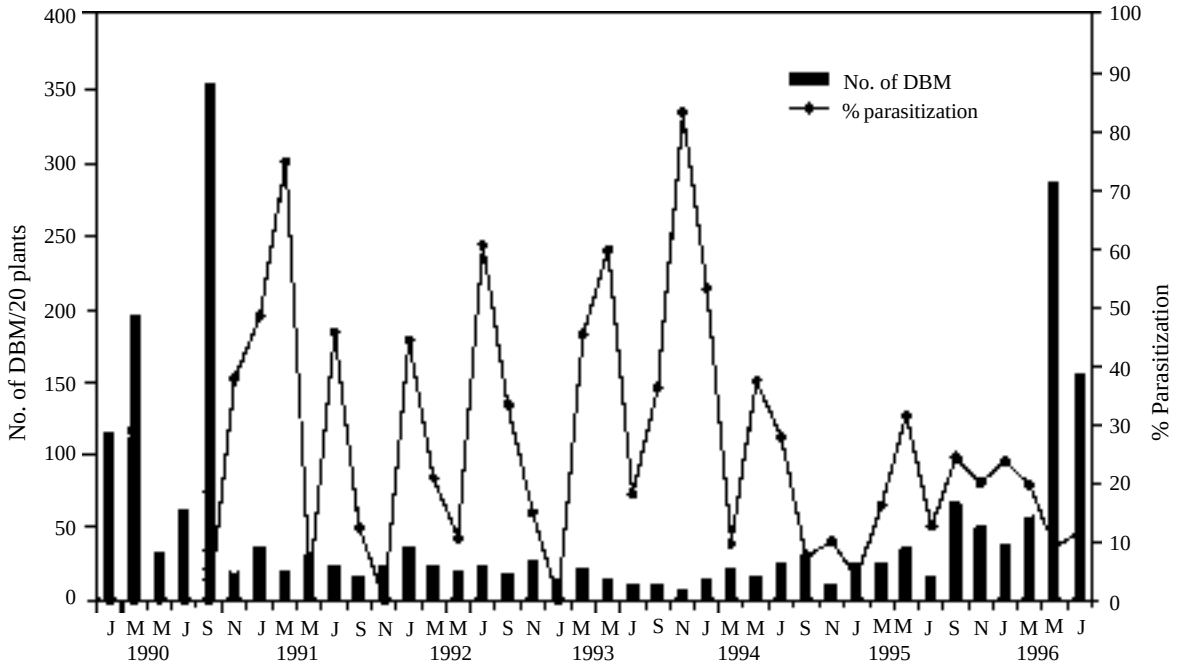


Figure 3. Population of DBM and % parasitization (Central Zone)

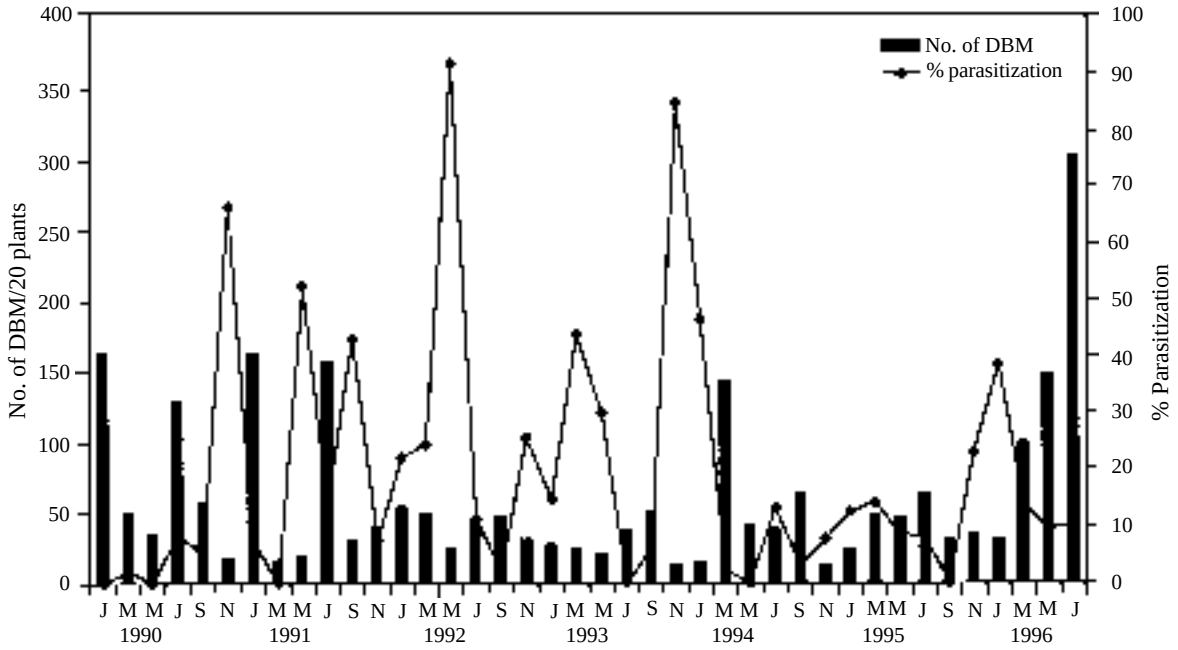


Figure 4. Population of DBM and % parasitization (Northern Zone)

B. thuringiensis subsp. *aizawai* and abamectin which are recommended insecticides in the IPM programme.

Second was introduction and use of new 'hard' insecticides by the growers in Cameron Highlands. A survey conducted by Arthurs (1996) showed use of 'hard' insecticides in combination with biological pesticides with increased frequency being commonly practised by a significant percentage of the cabbage growers in all regions of Cameron Highlands. This probably contributed to the reduction in parasitoid populations and development of resistance to the newly introduced insecticides. Arthurs (1996) also mentioned the use of unregistered insecticides by growers in Cameron Highlands which may have contributed to the ineffectiveness of 'newly' registered insecticides due to development of resistance long before these were sold in the market. This maybe true for the case of Fipronil which was only registered for use in 1996.

Thirdly, IPM activities (eg seminars, dialogues and training) with the farmers were very much reduced as compared to the periods 1989 to 1992. The lack of reenforcement of IPM and biological control probably caused the farmers to revert back to the 'old ways' to manage and control DBM. Taylor *et al.*, (1992) and Arthurs (1996) clearly showed that knowledge of IPM with farmers was more pronounced in the Central Zone than in the other areas, probably due to greater emphasis within this region as the research and extension centers are located in this region.

Table 1. The population of *Plutella xylostell*a and percent parasitization on cabbage in various zones in Cameron Highlands in 1990.

P. xylostella/Parasitoids	Zones		
	Southern	Central	Northern
P. xylostella (no./10 plants)	11.08	7.35	10.33
Cotesia plutellae (%)	11.90	7.55	10.33
Diadegma semiclausum (%)	3.95	27.27	5.33
Diadromus collaris (%)	0.55	4.53	4.60

Table 2. The population of *Plutella xylostella* and percent parasitization on cabbage in various zones in Cameron Highlands in 1995.

P. xylostella /Parasitoids	Zones		
	Southern	Central	Northern
P. xylostella (no./10 plants)	18.20	16.70	29.10
Cotesia plutellae (%)	9.00	2.80	2.80
Diadegma semiclausum (%)	10.40	17.00	5.30
Diadromus collaris (%)	0	0	0

Fourthly, the DBM-ETL in the IPM package, required tedious enumeration and very soon the growers became disinterested and reverted to weekly routine spraying of pesticides. There are some indications of this in the study by Arthurs (1996) even though a significant percentage of the growers used the ETLs when IPM was initially introduced (Taylor *et al.*, 1992). The possibilities of using sex pheromones of DBM as a monitoring tool was studied by Irfan U. (1996). However, the economics and feasibility of this method need further investigation.

Lastly, several studies (Taylor *et al*, 1992; Arthurs, 1996) showed that performance of the parasitoids varied in the different zones. In the Central Zone (elevation 1400–1500m) where temperatures are cooler, the level of parasitization was higher as shown in *Tables 1* and *2*. It is known that *D. semiclausum* performs more efficiently in cooler areas and in situations where population of DBM are low (Ooi, 1992). This could have attributed to the greater success of biological control and IPM in the Central Zone, and to a lower extent in the other areas.

Conclusion

In the last two decades research has led to the development and implementation of biological control of DBM in Cameron Highlands. The success of IPM (using biological control as the core) in Cameron Highlands can be attributed to two factors; firstly, the reduction in the use of synthetic insecticides and secondly, the increase reliance on parasitoids introduced specifically for DBM.

However, to maintain biological control and IPM, besides the reduction of insecticide usage, several other major factors should be considered. These are: (i) commitment by the authorities to sustain IPM, (ii) IPM to be made the policy in all crop protection programmes, (iii) periodic parasitoid releases in new vegetable areas, (iv) accreditation of vegetable growers using IPM and (v) development of an Insecticide Resistance Management (IRM) Programme for DBM as discussed by Verkerk *et al.* (1996).

References

Arthurs, S. P. (1996). A study on the diamondback moth and its parasitoids in the Cameron Highlands, Malaysia. MSc thesis submitted to the University of London for the Diploma of Imperial College. London. United Kindom. 98p

Corbett, G. H. and Padgen, H. T. (1941). A review of some recent entomological investigations and observations. *Malayan Agricultural Journal 29*, 347–375.

Ding, T. H., Vimala, P. and Yusof, S. (1981). An agroeconomic survey of vegetable farming in Peninsular Malaysia. MARDI. Selangor. Malaysia. 139p.

Hanada, T. (1987). Promotion of vegetable production in the Tropics, TARC. Japan. 86p.

Irfan, U. (1996). Monitoring and control of *Plutella xylostella* (L) with sex pheromones in Cameron Highlands. Ph.D. Thesis submitted to Universiti Kebangsaan Malaysia (UKM) Bangi Selangor. Malaysia. 203p.

Lim, K. P. (1970). Economics of vegetable market gardening in Cameron Highlands. M.Sc Thesis submited to University Malaya. Kuala Lumpur. Malaysia.

Lim, G. S. (1974). Integrated pest control in the developing countries of Asia. *In:* Environment and Development. Ed. D.M. Dworkin. Indianapolis, Scope Misc. Publ.

Lim, G. S. (1982). The biology and effects of parasites on the diamondback moth (*Plutella xylostella* (L). Ph.D. Thesis submitted to the University of London. United Kindom. 317p.

Lim, G. S., and Ko, W. W. (1975). *Apanteles plutellae* kurdj., A new recorded parasite of *Plutella xylostella* (L.) in Malaysia. *MARDI Res. Bull., 3:* 94–95.

Lim, G. S., Sivapragasam, A. and Ruwaida, M. (1986). Impact assessment of *Apanteles plutellae* on diamondback moth using an insecticide-check method. *In:* Talekar, N. S. and Griggs. T. D. (ed). Diamondback Moth Management. Proc. of the First International Workshop. AVRDC. Taiwan. 195–204.

Lim G. S., Loke, W. H., Chan, H. H. and Syed. A. R. (1988). Pest Management of Vegetables in Malaysia. Paper presented at the Informal Expert Consultation on IPM in Major vegetable crops in Asia. Nov. 14–16, 1988. FAO. RAPA. Bangkok. Thailand, 79p.

Loke, W. H. Dzolkhifli, O., Mohd. Rani, Y, and Sivapragasam, A. (1990). Exploring novel techniques for DBM Management. Seminar Pengurusan *Plutella xylostella* di Malaysia: Perspektif dan Strategi, 24 Aug. 1990. Kuala Lumpur. Malaysia.

Loke, W. H. Lim, G. S., Syed, A. R., Abd. Aziz, AM., Rani, M. Y., Md. Jusoh., M., Cheah, U. B. and Fauziah, I (1992a). Management of Diamondback Moth in Malaysia : Development, implementation and impact. In : Diamondback Moth and Other Crucifer Pests (ed. N.S. Talekar) AVRDC. Tainan. Taiwan : 529–539.

Loke, W. H., Sivapragasam, A., Syed, A. R. and Rani, M.Y. (1992b). Approaches to enhance biological control with parasitoids for IPM of DBM in crucifers. Workshop on Biological Control of *Plutella*. 19th Int. Congr. Entomol. 28 June - 4 July, 1992, Beijing, Peoples Republic China.

Ooi, P. A. C., (1979). Incidence of *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) and its parasite, *Apanteles plutellae* Kurdj. (Hymenoptera : Braconidae) in Cameron Highlands, *Malaysia*. *Malaysian Appl. Biol., 8:* 131–143.

Ooi, P. A. C. (1986). Diamondback moth in Malaysia. *In:* Talekar, N.S., and Griggs, T.D. (ed). Diamondback Moth Management : Proc. of the First international Workshop. AVRDC. Shanhua. Taiwan. 25–34.

Ooi, P. A. C. (1992). Role of Parasitoids in Managing Diamondback Moth in the Cameron Highlands, Malaysia. *In:* Talekar, N. S. (ed) Diamondback Moth and Other Crucifer Pests : Proc. of the 2nd International Workshop. AVRDC. Shanhua. Taiwan. 255–262.

Ooi, P. A. C. and Sudderuddin, K. J. (1978). Control of Diamondback Moth in Cameron Highlands, Malaysia. *In:* Proc. of Plant Protection Conference 1978. Rubber Research Institute of Malaysia. Kuala Lumpur. 214–227.

Ooi, P. A. C. and Lim. G. S. (1983). Introduction of exotic parasitoids to control the diamondback moth in Malaysia. Symposium on Res. in Biol. and Biotech. in Developing Countries, Singapore, 2–4 November, 1983.

Sudderuddin, K.I. and Kok, P.F. (1978). Insecticide resistance in *Plutella xylostella* collected from the Cameron Highlands of Malaysia. FAO Plant Protection Bull. 26. 53-57.

Syed. A. R. (1992). Insecticide Resistance in Diamondback Moth in Malaysia. *In:* Talekar, N. S. (ed) Diamondback Moth and Other Crucifer Pest: Proc. Of the 2nd International Workshop. AVRDC. Shanhua. Taiwan. 437–442.

Syed, A. R. and Loke, W. H. (1995). Development and challenges in pest management of vegetables: Seminar on Development and Challenges in Pest Management in Malaysia. 28 March 1995. Air Keroh. Melaka. MARDI and MAPPS. 33p.

Syed, A. R., Loke. W. H., Sivapragasam, A., Fauziah., I., Cheah., U. B., Md. Jusoh, M. Hussan, A. K. and Yusof, O. (1992). Proc. AVNET-1 Final Workshop. 23–26 Sept. 1992, Lembang, Indonesia.

Taylor, D. C., Zainal, A. M., Mad Nasir, S. Mohd Ghazali, M. and Chiew, E. F. C. (1992). Sustainability of English cabbage production practices in Cameron Highlands. Special Report Jabatan Ekonomi Pertanian. Universiti Pertanian Malaysia. 121p.

Verkerk, R. H. J., Syed. A. R. and Wright, D. J. (1996). The development of an insecticide resistance management programme for the diamondback moth in Malaysia. *In:* Proc. International Pesticide Conference: Crop Protection Towards 2000. 23–25 April 1996. MACA and GIFAP. Kuala Lumpur. 114–117.

Biological control of *Bemisia argentifolii* (Homoptera: Aleyrodidae) Silver leaf white fly; a crucifer pest in the southern USA

A. A. Kirk[1], M. A. Ciomperlik[2], J. A. Goolsby[2], D. C. Vacek[2], L. E. Wendel[2], J. C. Legaspi[3]

[1]*European Biological Control Laboratory, USDA/ARS, Parc Scientifique Agropolis, 34397, Montpellier, CX 5, France*

[2]*Mission Biocontrol Center, USDA/APHIS/PPQ, PO Box 2140, Mission, TX, 78873–2140, USA*

[3]*Texas Agricultural Experiment Station, 2415, East Highway 83, Weslaco, TX 78596*

Abstract

Since 1991 *Bemisia argentifolii*, the silverleaf whitefly (SLWF), has caused annual crop losses of $500 million in the USA including crucifer crops in California and Texas. Foreign exploration for (SLWF) natural enemies has been carried out in 25 countries including SE Spain where the agricultural system is similar to parts of Texas and California where (SLWF) is a pest. Parasitized (SLWF) were sent to the APHIS/PPQ quarantine, Mission Biological Control Center, TX, (MBCC) from Murcia Spain in 1991. The *Eretmocerus* sp. which emerged was identified using a RAPD-PCR technique and given a unique number (*Eretmocerus* nr. *mundus* M92014 - Spain) which distinguishes it from native and other exotic species of *Eretmocerus.* Evaluation in both the laboratory and in the Lower Rio Grande Valley on kale, and broccoli showed that this species parasitized more (SLWF) than the native *Eretmocerus* spp. and other exotic parasitoids tested. It has been recovered from (SLWF) infested cole crops at several release sites through 94–96. In addition M92014 is more tolerant of selected pesticides than native US *Eretmocerus* spp.

Key words: *Bemisia, Eretmocerus,* Spain, USA, Biocontrol, Crucifers

Introduction

Bemisia argentifolii Bellows & Perring, 1994, the Silverleaf Whitefly (SLWF) (Perring *et al,*1992) is a highly polyphagous, insecticide resistant *Bemisia* species recognised in the USA since 1988/89. Both *B. argentifolii* and the Diamondback moth *Plutella xylostella* (L.) are very major, region-wide introduced pests often occurring together in the southern USA where crucifers are an important component of the agricultural system. Both pests exhibit strong invasive potential; wide host plant ranges which make them a very serious threat to a broad range of crops; resistance to pesticides and in the case of *P. xylostella* resistance to *Bacillus thuringiensis*; migratory tendencies, leading to mass migrations of clouds of SLWF between crops; and to annual movement of *Plutella* northwards. Both have spread widely due to the intervention of man in moving infested cuttings and seedlings from one part of the country to another. *B. argentifolii* has been recorded from over 900 plant species in 74 families, (Cock, 1986, 1993). Outbreaks of *B. argentifolii* in Arizona, California, Florida, and Texas, caused estimated crop losses in excess of $500 million in 1992 (Faust & Coppedge, 1995). *B. argentifolii* was rarely seen on cruciferous crops before 1990 in the agricultural region of southern California (Perring *et al.* 1991) and did not constitute an economic threat. In 1990 reports indicated that cole crop growers were using multiple pesticide applications against whiteflies for the first time (Perring, *et al.* 1991) and that there was particularly severe damage to cauliflower and broccoli, *Brassica oleracea* L. In southern Texas severe damage to cabbage was also noted at the same time (Elsey & Farnham, 1994). In addition to considerable direct damage, cole crops act as overwintering reservoir plants for whitefly populations, which move onto cotton in spring when the crucifers are harvested. A number of physiological disorders associated with the presence of immature whitefly stages leading to weight loss have been recorded; in Hawaii yellowing and stem blanch in Kai Choy, (Costa, *et al.* 1993); and in 1990 in Arizona white streaking disorder of broccoli and cauliflower, (Brown *et al,* 1992) . The appearance of an apparently new gemini virus, Cabbage leaf curl virus followed the arrival of *B. argentifolii* in southern Florida (Abouzid *et al,* 1992) and Bedford *et al,*(1994a, b) report leaf yellowing on *B. argentifolii* infested cabbage and cauliflower. *B. argentifolii* is resistant to most insecticides, and despite massive pesticide spraying its numbers continue to increase. Besides causing rapid development of resistance to insecticides, massive pesticide treatments impact on non-target organisms leading to a severe reduction or elimination of natural enemies, further compounding the problem. Foreign exploration for natural enemies has been carried out by the USDA/ARS European Biological Control Laboratory (EBCL) based at

Montpellier, France since 1991 (Kirk *et al.*, 1993; Lacey *et al.* 1993). This paper reports on the collection, shipment, rearing, release and preliminary evaluation of insect parasitoids for the biological control of *B. argentifolii* in cole crops in the USA.

Materials and Methods

Natural enemy collections and shipments

Collection sites were selected, based on climate matching with whitefly infested areas in Arizona, California, Florida and Texas, Walter and Lieth (1967), Anonym. (1963). The diverse landscapes and agricultural systems present worldwide suggested a potential for many suitable habitats for whiteflies and natural enemies. Extensive collections were made twice in the same areas when possible, in spring and fall to cover the widest range of conditions. Parasitized whitefly populations were searched for on crop plants, weeds, trees, and ornamentals. Whitefly (parasitized) infested leaves were carefully removed from plants, air dried for 12 hours, wrapped in tissue paper, and placed in paper bags. Each sample bag was placed into another bag and finally a third, which was stapled shut. All samples were collected and stored separately, based upon host plant and locality. Collections were shipped in insulated boxes by airfreight to the USDA/APHIS/PPQ quarantine at Mission, TX (MBCC) where parasitoids emerged.

Rearing of natural enemy cultures

Natural enemies of *B. argentifolii* imported into the MBCC, were placed in cages containing *B. argentifolii* infested plants. Rearing conditions were 24–29 CX, 50–70% RH and a light regime of 14:10. A maximum number of unique species or biotypes of natural enemies were isolated into separate emergence containers by date, geographic location, and host plant. Individuals from each candidate population are collected from the emergence containers and used to start separate cultures. Cohorts are also collected for identification using classical taxonomic, biochemical and molecular genetic approaches.

Quarantine screening of natural enemies

Quarantine screening was initiated to estimate the potential effectiveness of exotic *Eretmocerus* and *Encarsia* spp. parasitoids in order to prioritize which species are mass reared for field release and evaluation (Goolsby *et al*. 1996). Screening for potential effectiveness is accomplished by measuring the reproductive potential and attack rate of individual female parasitoids on *B. argentifolii* infesting cole crops.

Identification and genetic markers for insect parasitoids

The identification of the hymenopterous parasitoids using PCR techniques has not previously been attempted and is an important tool in: maintaining the quality of parasitoid species colonies in quarantine; evaluation experiments; and the following up of the natural enemies released into the field. The DNA of individual hymenopteran parasites was amplified in a GeneAmp^{TM} PCR System 9600 thermal cycler with AmpliTaq^{R} DNA polymerase. The following primers were synthesized by Operon Technologies Inc. for use in this study: C04: 5'-CCGCATCTAC-3', C01: 5'-TTCGAGCCAG-3', BAM: 5'-ATGGATCCGC-3', and ECO: 5'-ATGAATTCGC-3'. Insect DNA isolation and purification, PCR reaction parameters, and electrophoresis of PCR products were according to Black *et al*. (1992). Representative gel patterns of the voucher specimens are stored on computer disks in the MBCC.

Classical identifications by systematists

Microscope slides of adult parasitoid specimens were prepared using the technique described by Noyes (1982), and of whitefly nymphal case remains using Martin's (1987) method. The whitefly were sent to S. Nakahara at the Systematic Entomology Laboratory, Beltsville, MD, USA and R. Gill, California Department of Agriculture, Sacramento, CA. The *Encarsia* were sent to M. Schauff at the Systematic Entomology Laboratory, Beltsville, MD, USA.and J. B. Woolley at Texas A&M University, College Station, TX. *Eretmocerus* species were sent to M. Rose and G. Zolnerowich at Texas A & M University, College Station, TX.

Field evaluation

Releases of the exotic parasitoids in broccoli at the Mission Biological Control Demonstration Farm totalled: 52,000 (M92014 *Eretmocerus* sp. - Spain), 54,000 for (M95012 *Eretmocerus* sp. - Pakistan) and 60,000 for (M94055 *Encarsia* sp. nr. *pergandiella* - Brazil) and 6000 of the (M95001 *Encarsia* sp. novo - Dominican Republic).

Results

Natural enemy collections and shipments

Between 1992–1996 more than one hundred shipments of SLWF natural enemies were sent to the MBCC by ARS European Biological Control Laboratory (EBCL) scientists and US and overseas collaborators (Lacey *et al*, 1993). Silverleaf whitefly infested plants in arid to wet climatic areas within different agricultural systems have been surveyed for SLWF natural enemies in 25 countries in Africa, S. America, the Mediterranean, Indian sub-continent and SE Asia once or several times. More than 98% of parasitoids emerged from SLWF and *Trialeurodes vaporariorum* (Westwood) (GHWF), infesting 50 plant hosts; 17 crops (mainly Cucurbitaceae/Solanaceae), 13 Ornamentals (no dominant family), 20 weeds (mainly Compositae/Solanaceae).

Identifications and genetic markers for insect parasitoids

Collaborators biotyped *Bemisia* populations from these diverse collections using esterase electromorphs. *B. argentifolii* was identified from Cyprus, France, Italy,

Spain, and Egypt. Fourteen *Encarsia* spp. of the 19 spp. recorded worldwide plus 7 new spp.; 1 confirmed *Eretmocerus* spp. of the 5 recorded plus 15 new spp, and 1 *Amitus* sp. were collected. The parasitoids at Mission were identified using a RAPD diagnostic method and given unique M numbers.

Releases and recoveries

More than 6 million *Eretmocerus* and *Encarsia* spp. from 24 cultures with 14 distinct DNA patterns were released in CA,AZ,TX,F,NH,NY. Three species have been chosen by the MBCC as key exotic natural enemies in the Rio Grande Valley TX Demonstration of Biological Control Based lPM program in collaboration with ARS, Weslaco: *Eretmocerus* sp. from southern Spain, *Eretmocerus* sp. from Pakistan and *Encarsia* nr. *pergandiella* from Brazil. Recoveries of these species have been made in AZ, CA and TX.

Field Evaluation

The combined results of two broccoli trials indicate that there are significant differences in the numbers attacked from the species tested ($F = 12.2$; $df = 17,302$; $P < .0001$). Of the species tested, *Eretmocerus* sp. nr. *mundus* (M92014) attacked significantly more *B. argentifolii* than the other species tested. The combined results of the two trials also confirm that many of the exotic parasitoids reproduce significantly better on crucifers than do the native species, which may afford the exotic species an advantage in the field. This is important because *B. argentifolii* overwinters in the field primarily on crucifers. In AZ and CA the native *Eretmocerus* sp. does not readily accept or develop in *Bemisia* on crucifers (Hoelmer, unpublished data). The field efficacy of *Eretmocerus* sp. nr. *mundus* (M92014) in crucifers was further evaluated in a field demonstration of Biological Control Based - IPM of B. *argentifolii* in Mission, TX. Percent parasitism was highest in the broccoli plots on the demonstration farm, followed by the kale refuge strip in the control farm. Densities of *B. argentifolii* immatures at the end of the season were significantly lower in the broccoli plots on the demonstration farm as compared to the kale refuge strips. Parasitized *B. argentifolii* from the plots were held for emergence of the adult parasitoids. In the control kale, all of the parasitoids reared from the samples were the native *Encarsia pergandiella* Howard. In comparison, the parasitoids reared from the demonstration broccoli plots were a mix of native *E. pergandiella* and exotic *Eretmocerus*. The exotic *Eretmocerus* were identified by Vacek using RAPD-PCR as *E.* nr. *mundus* (M92014-Spain). Although equivalent numbers of the exotic *Eretmocerus* sp. (M95012-Pakistan) were released in the broccoli, none were recovered. This result is consistent with prior quarantine screening and field cage tests which showed the *Eretmocerus* from Spain had significantly higher fecundity on broccoli than the *Eretmocerus* sp. from Pakistan.

Discussion

B. argentifolii overwinter on cruciferous crops in southern California and Texas, moving onto other crops such as melons rather quickly in spring after the crucifers are harvested. Pest control in The Lower Rio Grande Valley and southern Californian agroecosystems is predominately driven by pesticide applications. The *Eretmocerus* sp. from Spain was collected in am area where if anything chemical applications are more intense. *Eretmocerus mundus* from southern Spain was more tolerant of selected pesticides than *Eretmocerus* spp. from the USA (Jones *et al.* 1996). Because of this tolerance M92014-Spain can be introduced into these agroecosystems. Releases of exotic parasitoids in the demonstration broccoli plots increased levels of parasitism as compared to the kale refuge strips where no releases were made. *Eretmocerus* sp. M92014 from Spain appears to be largely responsible for the increase in parasitism and control of *B. argentifolii* in broccoli. Recovery data shows that the *Eretmocerus* sp. from Spain successfully overwinter on cole crops and field evaluation indicates that it is more effective in destroying *B. argentifolii* than the native *Eretmocerus* sp. in cole crops. The long term beneficial impact of this species in the Lower Rio Grande Valley agroecosystem, its dispersion and impact on *B. argentifolii* will be monitored using a RAPD molecular technique to detect it in field collected natural enemy populations.

References

Abouzid, A. M., Hiebert, E. and Strandberg, J.O. (1992). Cloning, identification, and partial sequencing of a geminivirus infecting the Brassicaceae. *Phytopathology* 82, abstract 7, 1070

Anonym. (1963) Ecological study of the Mediterranean zone. Bioclimatic map of the Mediterranean zone. 60 pp. *Recherches sur la zone aride* **XXI**, UNESCO-FAO, Paris

Bedford, I.D., Briddon, R.W., Brown, J.K., Rosell, R.C. and Markham, P. G. (1994a). Geminivirus transmission and biological characterization of *Bemisia tabaci* (Gennadius) biotypes from different geographic regions. *Annals of Applied Biology* 125, 311–325

Bedford, I.D., Pinner, M., LIU, S. and Markham, P. G. (1994b). *Bemisia tabaci* - potential infestation, phytotoxicity and virus transmission within European agriculture. Proceedings 1994 Brighton Crop Protection Conference- Pests and Diseases 1994, 911–916

Black, W. C., Duteau, N. M., Puterka, G.J., Nechols, J. R., and Pettorini, J. M. (1992) Use of the random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) to detect DNA polymorphisms in aphids (Homoptera: Aphididae). *Bulletin of Entomological Research 82*, 151–159

Brown, J.K., Costa, H.S. and Laemmlen, F. (1992). First report of whitefly-associated squash silverleaf disorder of *Cucurbita* in Arizona and of white streaking disorder of *Brassica* species in Arizona and California. *Plant Dis.* ***76:*** 426

Cock, M.J.W. (1986) *Bemisia tabaci*, a literature survey on the cotton whitefly with an annotated bibliography. *CAB IIBC*, Silwood Park, UK, 121 p.

Cock, M.J.W. (1993) *Bemisia tabaci*, an update 1986–1992 0n the cotton whitefly with an annotated bibliography. *CAB IIBC*, Ascot,UK, 78 pp.

Costa, H. S.,Ullman, D.E., Johnson, M.W. and Tabashnik, B.E. (1993). Association between *Bemisia tabaci* density and reduced growth, yellowing, and stem blanching of lettuce and Kai Choy. *Plant Disease* ***77(10):*** 969–972

Elsey, K. D. and Farnham, M.W. (1994) Response of *Brassica oleracea* L. to *Bemisia tabaci* (Gennadius) *HortScience* ***29(7):*** 814–817

Faust, R.M. and Coppedge, J.R. (1995) Foreword, 305 pp In: Henneberry, T.J., N.C. Toscano, R.M. Faust, J.R. Coppedge (Eds.) 1995 supplement to the 5-year National Research and Action Plan-Third Annual review, San Diego, CA, *USDA, ARS*, 1995–2

Goolsby, J., Legaspi, J.C. and Legaspi, B.C. (1996). Quarantine evaluation of exotic parasitoids of the sweetpotato whitefly, *Bemisia tabaci* (Gennadius). *South-west Entomologist* ***21(1):*** 13–21

Jones, W. A., Wolfenbarger, D.F. and Kirk, A.A. (1996). Response of adult parasitoids of the sweetpotato whitefly *Bemisia tabaci* (Hom:Aleyrodidae) to leaf residues of selected cotton insecticides. *Entomophaga* 40: 153-163

Kirk, A.A., Lacey, L. A., Roditakis, N. and Brown, J.K. (1993). The status of *Bemisia tabaci* (Hom:Aleyrodidae), *Trialeurodes vaporariorum* (Hom:Aleyrodidae) and their natural enemies in Crete. *Entomophaga* 31, 183–190

Lacey, L.A., Kirk, A.A. and Hennessey, R.D. (1993). Foreign exploration for natural enemies of *Bemisia tabaci* and implementation in integrated control programs in the United States. III *Proceedings Association National de Protection des Plantes*, 1, 351–360

Martin, J.H. (1987) An identification guide to common whitefly pest species of the world (Homoptera, Aleyrodidae). *Tropical Pest Management.* ***33:*** 298–322

Noyes, J.S. (1982) Collecting and preserving chalcid wasps (Hymenoptera: Chalcidoidea). *Journal of Natural History*. ***16:*** 315–334

Perring, T. M., Cooper, A., Kazmer, D. J., Shields, C., and Shields, J. (1991). New strain of sweetpotato whitefly invades California vegetables. *California Agriculture* 45, 10–12

Perring, T.M., Cooper, A., and Kazmer, D. J. (1992). Identification of the Poinsettia strain of *Bemisia tabaci* (Homoptera: Aleyrodidae) on broccoli by electrophoresis. *J. Econ. Entomol.* ***85:*** 1278–1284

Walter, H. and Lieth, H. (1967) *Klimadiagram-Weltatlas*.– Gustav Fisher Verlag, Jena, Germany

Host specificity assessments of *Cotesia plutellae*, a parasitoid of diamondback moth

P.J. Cameron[1], G.P. Walker[1], M.A. Keller[2] and J.R. Clearwater[3]

[1]*New Zealand Institute for Crop & Food Research Ltd, Private Bag 92169, Auckland, New Zealand*

[2]*Department of Crop Protection, University of Adelaide, South Australia 5064*

[3]*Horticulture and Food Research Institute of New Zealand, Private Bag 92169, Auckland, New Zealand. Current address, 63 Peter Buck Rd, Auckland.*

Abstract

Cotesia plutellae is being assessed as a potential biological control agent for introduction against *Plutella xylostella* in New Zealand. As the literature on *C. plutellae* provided variable assessments of its host specificity, further information was collected from the laboratory and field. Our field assessments in Fiji indicated that this parasitoid did not attack other Lepidoptera in or around vegetable brassica crops. Laboratory tests on a colony of *C. plutellae* in South Australia, including simple no-choice experiments and flight tunnel choice tests, showed that the parasitoid could choose to oviposit in other Lepidoptera and that successful development rarely occurred. In New Zealand, similar laboratory tests of *C. plutellae* collected from Fiji revealed that it was capable of ovipositing and developing in seven other species of Lepidoptera. Host suitability was assessed by comparing the ability of the parasitoid to develop in *P. xylostella* and other species. Host acceptability was compared by assessing the flight of adults to test larvae on their host plants, and by comparing oviposition preferences. These experiments suggested that *C. plutellae* may parasitise Lepidoptera other than *P. xylostella* in New Zealand and indicate that further assessments are required to determine its potential impact in the field.

Key words: *Cotesia plutellae, Plutella xylostella*, host specificity, oviposition, development.

Introduction

Cotesia plutellae Kurdjumov (Hymenoptera: Braconidae) is being considered as a candidate for introduction into New Zealand to augment the existing parasitoids of diamondback moth (DBM) *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae). These parasitoids, *Diadegma semiclausum* (Hellen) and *Diadromus collaris* (Gravenhorst), can provide high rates of parasitism in the spring and early summer, but in dry periods of the summer DBM population increases are often not controlled by existing natural enemies. Insecticide applications are then necessary (Beck and Cameron, 1992). *C. plutellae* was proposed for introduction because it kills DBM larvae at an earlier stage than the existing parasitoids and therefore may reduce feeding damage on host plants. This parasitoid may also supress DBM better than *D. semiclausu*m because it is more active at higher temperatures (Talekar and Yang, 1991).

The major question concerning the introduction of *C. plutellae* into New Zealand is its specificity to DBM and its possible effects on non-target species. Fitton and Walker (1992) point out that although *C. plutellae* is widely assumed to be host specific, it has been recorded from several other species of Lepidoptera. Although for some early biological control introductions native alternative hosts were considered as useful reservoirs (Cameron *et al.* 1993), conservation of native species, including the few attractive native butterflies in New Zealand, is now an important issue. In this study, our assessments of the host specificity of *C. plutellae* have included consideration of the literature, assessments of its host range in the field, and laboratory experiments to define behavioural and physiological measures of host range.

Methods

Field survey

The specificity of *C. plutellae* in the field was examined by collecting and rearing Lepidoptera larvae from crucifers and adjacent weeds in areas of Fiji where the parasitoid was known to be present (Walker *et al.*, in press). Larvae were reared individually to allow any parasitoids to emerge, and representative Lepidoptera larvae and adults were retained for identification. Dr J.A. Berry and A.K. Walker identified the hymenopterous parasitoids and J.S. Dugdale identified the Lepidoptera. Similar observations were carried out in South Australia, although the apparent absence of *C. plutellae* minimised their value.

Laboratory assessments

Sources of insects: Laboratory experiments were performed in 1994 at the University of Adelaide with a culture of *C. plutellae* that had been obtained from Dr N.S. Talekar in Taiwan. Separate experiments were carried out at Crop & Food Research in Auckland with *C. plutellae* collected from Fiji in 1995. Both cultures were identified by Drs J.A. Berry and A.D. Austin, and voucher specimens have been deposited in the New Zealand Arthropod Collection at Landcare Research in Auckland.

C. plutellae was reared on DBM larvae fed on cabbage. Adult parasitoids were held in 4 litre, ventilated, clear plastic containers at 21 °C and fed dilute honey solution on cotton wicks. From days 2 to 5 after adult emergence, approximately 60, 2nd and 3rd instar larvae on a small cabbage leaf were presented for parasitisation to 10 females (and a similar number of males) for 3 h. The larvae were then reared on cabbage, and parasitoid cocoons were collected after 11–13 days and if necessary stored at 15 °C in glass vials for up to 1 week.

Lepidoptera species to be tested were generally collected as adults from light traps. Eggs were collected from gravid females and larvae reared on their usual host plant or on cabbage. The majority of test insects were collected from Auckland *(Table 1)*; those from other sources were: *Plutella antiphona Meyrick* (from Chatham Island), Australian *Bassaris itea* F. (Adelaide, South Australia), Australian *Diarsia intermixta* Guenee (Devonport, Tasmania), *Neumichtis saliaris* Guenee (Devonport, Tasmania).

Host suitability: The suitability of Lepidoptera larvae of different species for development of *C. plutellae* was tested by exposing individual larvae to a single mated female in a 100 x 25 mm glass vial for 5 minutes in an attempt to force oviposition. Larvae were chosen to match the approximate size of the 2nd or 3rd instar *P. xylostella* used as controls. Larvae were presented to one female in a sequence, alternating control and test larvae, until the parasitoid failed to oviposit in DBM. The time taken to initiate oviposition was also measured. The success of oviposition was checked by dissecting some test larvae after 48 h to determine if eggs had been deposited or larvae were developing. The remaining test larvae were reared until parasitoid larvae emerged to form cocoons, or until the test larva died. The comparative success of parasitoid development in different test species was also assessed by exposing six replicates of 8–12 test larvae to individual females in 4 litre cages for 3 h. In similar experiments, parasitoid females were provided with a choice between larvae of DBM or another test species. The results were expressed as the number of *C. plutellae* cocoons per number of larvae exposed to parasitism.

Flight tunnel tests: The acceptability of different test species was assessed by observing the flight of adult female *C. plutellae* to larvae on excised leaves in a flight tunnel. These behavioural tests were initiated at the University of Adelaide using methods developed by Keller (1990). The wind speed in the tunnel was set at 60 cm/s. Adult parasitoids were released at 70 cm from the test insects, and the experiments were run at 25 °C. At Crop & Food Research in Auckland, behavioural assays were continued using a flight tunnel based on the design of Miller and Roelofs (1978). The tunnel was operated at a wind speed of 50 cm/s and a temperature of 21 °C. Test females were fed and mated but had no experience of Lepidoptera larvae prior to release in the tunnel. Females were presented with larva-plant combinations either alternately (no-choice) or simultaneously (choice test). Five to ten test insects were placed on each plant 24 h prior to the experiments to ensure the presence of some leaf damage. Plants were presented as one or two excised leaves to provide a similar leaf area for each test. For the choice tests, the plants were placed approximately 15 cm apart

Table 1. Oviposition and development of *Cotesia plutellae* in test insects, measured as number per larvae exposed to parasitism

Family Test insect	Ovipositions per exposure	Eggs per oviposition	Cocoons per oviposition	Cocoons per exposure
Yponomeutidae				
Plutella xylostella (DBM)	182/190	32/41	21/23	60/110
Plutella antiphona	–	–	7/8	21/72
Tortricidae				
Epiphyas postvittana	6/33	0/1	0/5	0/48
Pieridae				
Pieris rapae	11/32	0/11	0/3	0/60
Nymphalidae				
Basaris itea	5/40	–	1/12	–
Basaris itea ex Australia	0/30	–	–	–
Arctiidae				
Nyctemera amica	17/24	–	–	7/22
Noctuidae				
Agrotis ipsilon	–	0/3	5/12	1/50
Diarsia intermixta	–	2/6	1/8	9/50
Diarsia intermixta ex Australia	–	–	2/31	–
Graphania mutans	15/30	–	3/17	3/50
Graphania ustistriga	–	–	–	30/50
Helicoverpa armigera	16/30	2/16	0/5	–
Neumichtis saliaris ex Australia	–	–	0/20	–
Spodoptera litura	14/24	1/8	0/17	0/47
Thysanoplusia orichalcea	4/10	–	0/4	–

across the air flow, equidistant from the centre line, and their position was alternated between each test.

Results and Discussion

Published host records

Numerous field records suggest that *C. plutellae* is a narrowly oligophagous parasitoid of DBM that occasionally parasitises other Lepidoptera species. The majority of records in Shenefelt's 1972 summary of host records report DBM as the only host. The main exception to this is the extensive list of hosts derived from Wilkinson (1939) in his description of the parasitoid. Most of Wilkinson's records appear to be based on host identifications associated with parasitoid specimens, and six of the hosts are based on single records. After extensive rearing, Delucchi *et al.* (1954) considered that two of the species on Wilkinson's list, *Pieris rapae* L. and *Pieris brassicae* L., were not attacked by *C. plutellae*. In his surveys of parasitism of *Hyphantria cunea* Drury (Arctiidae), Bogavic (1953) recorded approximately 26 *C. plutellae*, equivalent to less than 0.01% parasitism. Together with Wilkinson's records of five parasitised *Diacrisia urticae* Esp. (Arctiidae), these records suggested that further host assessments should include representatives of this family. Wilkinson's (1939) report of 28 host records for two species of Lymantriidae and six host records for *Malacosoma castrensis* L. (Lasiocampidae) also warrant consideration. The most definite information on host association is that for *Aglais urticae* L. (Nymphalidae). Wilkinson (1939) received specimens of *C. plutellae* reared from *A. urticae*, and maintained a culture of this parasitoid on both DBM and *A. urticae*. He stated that "*.... Apanteles plutellae* Kurdj., is able to utilise both *P. maculipennis* and *A. urticae* as a host." The potential for rearing *C. plutellae* on alternative hosts in the laboratory was also demonstrated by Wang *et al.* (1972) who showed that although the parasitoid preferred to oviposit in DBM it survived better in the rice moth *Corcyra cephalonica* Stainton. Similarly, Lim (1982) (cited in Waterhouse and Norris, 1987) recorded that *Crocidolomia binotalis* Zeller and *Hellula undalis* Guenee were parasitised in the laboratory but not in the field. An additional field host record for *C. plutellae* was reported by Baloch *et al.* (1966) who recorded low rates of parasitism when assessing the potential of *Oeobia (=Anania) verbascalis* Schiff. (Pyralidae) for biological control of Noogoora burr, *Xanthium strumarium* L.

These literature records, together with general criteria for selecting test species, suggested three categories of test species:

1. Close relatives, i.e. *Plutellidae*.
2. Species on crucifers, especially Noctuidae.
3. Species in the same family as hosts recorded in the literature, i.e. *Nymphalidae, Noctuidae, Arctiidae, Pyralidae, Lymantridae, Lasiocampidae*.

Field survey

Collection and rearing *P. xylostella* in the Suva region of Fiji in 1992 and 1995 indicated that *C. plutellae* was common. It parasitised 74% of DBM in the 1995 survey (Walker *et al.* in press). Larvae of several Noctuidae and one Pyralidae were also collected from brassicas and weeds in the same area. Although other parasitoids were present, no *C. plutellae* were reared from 563 *Spodoptera litura* F., 43 *Helicoverpa armigera* (Hubner), 17 *Chrysodeixis eriosoma* Doubleday (all Noctuidae) and 130 *Hymenia recurvalis* (F.) (Pyralidae). *Crocidolomia binotalis* (Pyralidae) was similarly not parasitised, but the presence of *C. plutellae* in the region that this lepidopteran was collected was not confirmed. These results augment the previous observations of Lim (1982) (cited in Waterhouse and Norris, 1987) that *C. binotalis* and *Hellula undalis* were parasitised in the laboratory but not in the field.

Laboratory assessments

Test species: Initial assessments in South Australia in 1995 tested three native species. Two of these species, *Diarsia intermixta* and *Neumichtis saliaris* (Noctuidae), are associated with brassicas (Common, 1990), whereas the yellow admiral *Bassaris itea* (Nymphalidae) occurs on nettle *(Urtica dioica)* and is valued, particularly in New Zealand, as an attractive species. Test species in New Zealand included a near relative of DBM, the endemic species *Plutella antiphona*, which has a limited distribution on Cruciferae, particularly water cress, *Nasturtium officinale* (Dugdale, 1973). Seven test species that occur on brassicas in association with *P. xylostella* were Noctuidae *(Table 1)*. Other test species from brassicas were *Pieris rapae* (Pieridae) and *Epiphyas postvittana* (Tortricidae). The remaining test species where *Nyctemera amica* (Arctiidae) collected from ragwort *(Senecio jacobaea)* and *B. itea* (Nymphalidae), both representing families that include hosts of *C. plutellae* previously reported in the literature (Wilkinson, 1939). There are no Lasiocampidae or Lymantriidae in New Zealand so assessments of species from these families were not relevant.

Host suitability: In experiments with the University of Adelaide culture of *C. plutellae*, the parasitoid was induced to oviposit in *D. intermixta*, and from 31 oviposition attempts two cocoons were produced *(Table 1)*. Oviposition responses to *N. saliaris* were more difficult to obtain and no cocoons were produced from 20 oviposition attempts on this species. *B. itea* was unacceptable to *C. plutellae* to the extent that no oviposition responses were obtained. *C. plutellae* was collected from Fiji and imported for assessment in quarantine in Auckland. This culture attempted to oviposit in all test species, but dissection of larvae revealed that eggs were not deposited in *E. postvittana* or *P. rapae (Table 1)*. The oviposition rate was highest in DBM and oviposition was initiated more quickly (data not shown) in this species. There was no clear difference between oviposition rates in

species other than *P. xylostella*, nor was the oviposition rate related to success in cocoon formation. For example, no cocoons developed from *Spodoptera litura*, but more than 50% of the larvae attracted oviposition attempts. This demonstrated that oviposition provided a poor estimate of the suitability of a species, possibly because this response may be elicited by plant (cabbage)-associated factors. Of those species where eggs were detected, both *S. litura* and *Helicoverpa armigera* were unsuitable for further development. The rate of cocoon formation *(Table 1)* indicated that six species were not hosts: *B. itea, A. ipsilon* and *G. mutans* were occasional laboratory hosts; and *G. ustistriga, N. amica, P. antiphona* and *D. intermixta* were all suitable laboratory hosts for *C. plutellae*. Exposure of test species in choice tests reduced the rate of cocoon formation *(Table 2)* indicating that mixed host populations interfered with host location and reduced the probability of attack on alternate species.

Estimates of the development rate of *C. plutellae* provided another measure of the suitability of some test species. In *P. antiphona*, parasitoids developed from egg to cocoon at the same rate as in DBM. By contrast, *D. intermixta* and *G. ustistriga* required two more days to develop, and *N. amica* took four more days than DBM.

Flight tunnel tests: Flight tunnel experiments showed that *C. plutellae* females could fly to all test combinations of insect and plant species *(Table 3)*. During successful flights females frequently exhibited characteristic casting behaviour as they sampled the odour gradients produced by each source. In no-choice tests, fewer adults flew to *B. itea* on nettle, but plants with larvae of *H. armigera* or *N. amica* were as attractive as DBM. The rate of successful flights declined slightly in choice tests, but apart from reduced flights to *B. itea, C. plutellae* showed no distinct preference. Flights to *H. armigera* and *N. saliaris*, previously demonstrated to be unsuitable for development, strongly suggested that *C. plutellae* is attracted by cabbage volatiles, or by the volatiles from damaged cabbage. This behaviour has also been observed in *Cotesia rubecula* by Agelopoulos and Keller (1994a, b) who reported that, although this parasitoid did not distinguish between damage by host or non-host Lepidoptera, the blend of volatiles emitted from frass was different for DBM and *Pieris rapae*.

The development of behavioural measures that reflect the host range of oligophagous species such as *C. plutellae* will require further refinements of testing procedures. Whereas simple tests may be suitable for demonstrating high degrees of specificity such as found in *C. rubecula* (Cameron and Walker, in this proceedings), the specificity of *C. plutellae* is apparently determined more by behaviour than physiological compatibility. Testing procedures will therefore need to consider that because confinement disturbs the natural behaviour of parasitoids, laboratory tests will usually overestimate their host range in the field (Sands, 1993). To obtain more realistic measures of host range in the field, we also plan to extend our collection and rearing of Lepidoptera from regions where *C. plutellae* is naturally present.

Acknowledgements
We thank Anne Barrington, Tim Herman, Lionel Hill, John LeSueur and Chris Winks for supplying test

Table 2. Development of *Cotesia plutellae* cocoons from tests insects compared with *Plutella xylostella* (DBM) in choice and no-choice experiments, measured as number of cocoons per larvae exposed to parasitism

Alternate test insect and plant	Choice		No choice	
	Alternate	DBM on cabbage	Alternate	DBM on cabbage
Graphania mutans on cabbage	1/60	16/60	3/50	
Nyctemera amica on ragwort	9/60	16/60	7/22	41/50
Spodoptera litura on cabbage	0/50	12/50	0/47	

Table 3. Flights of *Cotesia plutellae* to test insect and host plant combinations compared with *Plutella xylostella* (DBM) in a flight tunnel

Alternate test insect and host plant	Number of flights per number of tests	
	DBM on cabbage	Alternate test combination
No choice test		
Bassaris itea on nettle	10/18	4/15
Helicoverpa armigera on cabbage	10/19	9/21
Nyctemera amica on ragwort	7/15	5/13
Choice test		
Bassaris itea on nettle	18/43	6/43
Nyctemera amica on ragwort	8/27	6/27
Diarsia intermixta on cabbage ex Australia	19/51	21/51
Neumichtis saliaris on cabbage ex Australia	13/30	14/30

insects, and Darryl Jackman and Sarah Painter for rearing various Lepidoptera and assisting with experiments. Andy Austin, Jo Berry and Annette Walker provided identifications of the parasitic Hymenoptera, and John Dugdale and Lionel Hill identified the Lepidoptera.

References

Agelopoulos, N.G. and Keller, M.A. (1994a). Plant-natural enemy association in the tri-trophic system *Cotesia rubecula-Pieris rapae*-Brassicaceae (Cruciferae) II: Preference of *C. rubecula* for landing and searching. *Journal of Chemical Ecology* ***20:*** 1 735–48.

—— (1994b). Plant-natural enemy association in the tri-trophic system *Cotesia rubecula-Pieris rapae*-Brassicaceae (Cruciferae) III: Collection and identification of plant and frass volatiles. *Journal of Chemical Ecology* ***20:*** 1 955–67.

Beck, N.G. and Cameron, P.J. (1992). Developing a reduced spray program for brassicas in New Zealand. In Diamondback moth and other crucifer pests (ed. N.S. Talekar) pp. 341–350.

Baloch, G.M., Din, I.M. and Ghani, M.A. (1966). Biology and host-plant range of *Oeobia verbascalis* Schiff. (Pyralidae: Lepidoptera); an enemy of *Xanthium strumarium* L. *Technical Bulletin of the Commonwealth Institute of Biological Control* ***7:*** 81–90.

Bogavic, M. (1953). Some observations on fall webworm parasites. *Zastita Bilja* ***16:*** 58–80.

Cameron, P.J., Hill, R.L., Bain, J. and Thomas, W.P. (1993). Analysis of importations for biological control of insects pests and weeds in New Zealand. *Biocontrol science and technology* ***3:*** 387–404.

Cameron, P.J. and Walker, G.P. (1997). Introduction and evaluation of *Cotesia plutellae*, a parasitoid of *Pieris rapae* in New Zealand. In: A. Sivapragasam, W.H. Loke, A.K. Hussan and G.S. Lim (eds.). Proceedings of the Third International Workshop on Diamondback Moth. Kuala Lumpur, Malaysia.

Common, I.F.B. (1990). Moths of Australia. E.J. Brill, The Netherlands, 535 pp.

Delucchi, V., Tadic, M. and Bogavic, M. (1954). Mass rearing of *Apanteles plutellae* Kurdj. (Hym., Ichneumonidae), endophagous parasites of *Plutella maculipennis* Curt. and biological observations on these parasites. *Zastita Bilja* ***21:*** 26–41

Dugdale, J.S. (1973). The genus *Plutella* (Hyponomeutidae) in New Zealand and the family position of *Circoxena* (Lepidoptera). *New Zealand Journal of Science* ***16:*** 1 009–23.

Fitton, M. and Walker R, A. (1992). Hymenopterous parasitoids associated with diamondback moth: the taxonomic dilemma. In Diamondback moth and other crucifer pests (ed. N.S. Talekar) pp. 225–232.

Keller, M.A. (1990). Responses of the parasitoid *Cotesia rubecula* to its host *Pieris rapae* in a flight tunnel. *Entomologia Experimentalis et Applicata* ***57:*** 243–249.

Miller, J.R. and Roelofs, W.L. (1978). Sustained-flight tunnel for measuring insect responses to wind-borne sex pheromones. *Journal of Chemical Ecology* ***6:*** 187–198.

Sands, D.P.A. (1993). Effects of confinement on parasitoid/host interactions: Interpretation and assessment for biological control of arthropod pests. In Pest Control and Sustainable Agriculture (ed S.A. Corey, D.J. Dall and W.M. Milne) pp. 196–199.

Shenefelt, R.D. (1972). Hymenopterum catalogus. Part 7, Braconidae 4. Dr W. Junk N.V s-Gravenhage.

Talekar, N.S. and Yang, J.C. (1991). Characteristics of parasitism of diamondback moth by two larval parasites. *Entomophaga* ***36:*** 95–104.

Walker, G.P., Cameron, P.J., Berry, J.A. and Lal, S.N. (in press). Parasitoids reared from lepidopteran larvae from brassicas and associated weeds in Fiji, 1992 and 1995. Proceedings of the Second Workshop on Biological Control in the Pacific.

Wang, C-L., Chio, H. and Ho, K-K. (1972). The comparative study of parasitic potential of the braconid wasp (*Apanteles plutellae* Kurdj.) to the diamondback moth (*Plutella xylostella* L.) and rice moth (*Corcyra cephalonica* Staint.). *Plant Protection Bulletin (Taipei)* ***14***: 125–8. (In Chinese, English summary).

Waterhouse, D.F. and Norris, K.R. (1987). Biological Control-Pacific Prospects. Inkata Press, Melbourne, 454 pp.

Wilkinson, D.S. (1939). On two species of *Apanteles* (Hym. Brac.) not previously recognised from the Western Palearctic region. *Bulletin of Entomological Research* ***30:*** 77–84.

Effects of nectar-producing plants on *Diadegma insulare* (Cresson), a biological control agent of diamondback moth, *Plutella xylostella* (L.)

Idris, A.B.[1] and Grafius, E.[2]

[1]*Department of Zoology, Faculty of Life Sciences, National University of Malaysia 43600 UKM, Bangi, Selangor Darul Ehsan, Malaysia*

[2]*Department of Entomology, Natural Science Building, Michigan State University East Lansing, MI 48824-1115, USA*

Abstract

Effects of nectar-producing plants (NPP) on the longevity, fecundity, oviposition and nectar-collecting behaviour of *Diadegma insulare* were studied. Longevity and fecundity of *D. insulare* were varied with morphological characters of NPP's flowers. *Brassica kaber* (D.C) Wheeler, *Barbarea vulgaris* R. Br. and *Daucus carota* L. supplied nectar and resulted in *D. insulare* longevity and fecundity equal to when honey+water used as food sources. Others were not significantly better than no food at all. *Chenopodium album* L. and *Thlaspi arvense* L. did not provide available nectar, however, adults parasitoid fed on honeydew excreted by aphids feeding on the plants. An increase in longevity and fecundity was correlated with flower corolla opening diameter but not with corolla length. The oviposition behaviour within the first minute of exposure to diamondback moth larvae was highly correlated with longevity and fecundity of *D. insulare*, which we considered indices of food quality. We observed five nectar-collecting behaviours of *D. insulare*. The most striking behaviour, on *B. vulgaris* and *B. napus* L. flowers, involved chewing at the base of the corolla and creating holes that probably released the floral nectars. *D. insulare* visit more frequently and spent longer time particularly at the base of flower supporting longer life and high fecundity. The charactersitics of NPP's flower and behavioural flexibility of *D. insulare* should be manipulated to increase its impact in integrated diamondback moth management.

Key words: *Plutella xylostella, Diadegma insulare*, nectar-producing plants, food sources

Introduction

Diamondback moth (DBM), *Plutella xylostella* (L.), is the major pest of *Brassica* crop worldwide. It possess the ability to develop resistance to all pesticides used against them (Tabashnik *et al.*, 1991). Pesticides resistance problems have forced growers to increase the frequency and rate of spray, and continue using whatever available pesticides to control DBM. This leads to excessive and indiscriminate use of pesticides that destroys the DBM natural biocontrol agents, especially the parasitoids and predators, in *Brassica* crops agroecosystem (Lim *et al.*, 1986). *Diadegma insulare* (Cresson) is a major DBM parasitoid in Canada (Harcourt, 1986) and United States of America (Idris and Grafius, 1993b). Judicious use of pesticides and good *Brassica* ecosystem management should be adopted because pesticides is detrimental to *D. insulare* (Srinivasan and Krishna Moorthy, 1991; Idris and Grafius, 1993a and b).

Earlier studies indicated that the presence of nectar-producing plants (NPP) in the field provides an important food source for the parasitoid which directly increases their effectiveness (van Emden, 1963; Leius, 1967; Keven, 1973; Syme, 1975). For *D. insulare*, Zhao *et al.* (1992) found that parasitism of DBM by this parasitoid was higher in the broccoli adjacent to NPP than in the broccoli that was not surrounded by NPP. In England, *Diadegma* species were observed feeding on the flowers of weeds in the vicinity of the field (Fitton and Walker, 1992). The selective use of floral nectar resources by the parasitoids was reported by Cowgill *et al.* (1993) and Jervis *et al.* (1993). An understanding of the relative importance of NPP to *D. insulare* may be important if we want to enhance its role and effectiveness in DBM management.

The objectives of our study were to assess the effects of NPP on the longevity and fecundity, oviposition and nectar-collecting behaviour of *D. insulare*, and to correlate flower structures with *D. insulare* and fecundity.

Material and Methods

Sources of NPP. Flowers of 8 Brassicaceous weeds; *Barbarea vulgaris* R. Br., *Berteroa incana* (L.) D.C., *Brassica kaber* (D.C.) Wheeler, *Brassica napus* L., *Capsella bursa-pastoris* (L.) Medic., *Erysimum cheiranthoides* L., *Lepidium campestre* (L.) R. Br. and *Thlaspi arvense* L.; 5 non-Brassicacaeae, *Chrysanthemum leucanthemum* L. and *Sonchus arvensis* L. (Asteraceae), *Rumex crispus* L. (Polygonaceae), *Chenoppodium album* L. (Chenopodiaceae),and *Daucus carota* L. (Umbelliferae), and one cultivated *Brassica* plant (canola) were used as nectar sources for the parasitoid. *Brassica* weeds were emphasized because they were

common in and near cabbage fields. They are also potential hosts for DBM larvae and are tolerant to many herbicides used in cole crops.

Sources of insects and site of study. We used $F_{18–20}$ DBM (Geneva strain) reared in the laboratory on broccoli leaves grown in the greenhouse, and $F_{2–3}$ field collected *D. insulare* reared on DBM. Study was conducted at the Michigan State University Entomology Research Farm in May through September, 1993 using NPP species available during each month.

Longevity and Fecundity. The flowers of NPP and *D. insulare* were enclosed in a cylindrical screen cage (20 cm high and 10 cm diameter) with sponge covering the top and bottom of the cage and small slit at the side of the screen for introducing insects. We cut a 5 cm slit from the edge to the center of the bottom for the flower stem(s). Each cage was tied to a wooden stake erected close to individual flowering weeds. The cage was moved to a new flower when the earlier flower began to wilt. For honey+water (10% honey) treatment and water alone treatments, filled in the glass vials (21 by 70 mm) the change were made every every 4-d. A piece of tissue paper was dipped into the vial to avoid excessive evaporation. We inserted the vial through a hole in the bottom foam. In September, two branches of the *C. album* and *S. arvensis* (naturally infested by bean aphids, *Aphis fabae* Scopli) with aphids and without aphids were inserted into the cage in place of the flowers. One male-female pair of *D. insulare* (1-d old and not yet fed) was released into the cage through a slit on the side of the screen. The treatments, including the no food, were replicated 8 times. Survival of the *D. insulare* females was recorded daily to measure the longevity. To measure fecundity, the adult female parasitoid were removed out from the cage (1100 to 1450 h, during which females are most active) (Idris,1995) every 3-d (started on the 3rd day after it was released into the cage) and released it into a 400-ml transparent plastic container with a screen lid with 30 3rd-instar DBM larvae for 3 h before putting it back into the cage. The presumably parasitized DBM larvae reared in the laboratory on broccoli foliage until pupation, when the number of *D. insulare* and DBM pupae were recorded. Fecundity was calculated as the sum of all *D. insulare* pupae produced by a female *D. insulare* during her life (30 host larvae offered every 3-d).

Ovipositional behaviour. On day 9, 4 of the 8 replicates for *D. carota, B. kaber , B. incana, C. bursa-pastoris, B. napus* and *T. arvense,* and honey+water treatments were observed in the above study and recorded oviposition behaviour (any attack on host made by the parasitoid that ended with inserting its ovipositor into host body) of *D. insulare,* within 1, 5, 20 min of exposure to DBM larvae.

Relationship between flower structure with D. insulare longevity and fecundity. The corolla length and diameter of the opening for a sample of 10 flowers (selected randomly) for each species per replicate were measured and used to relate it with the longevity and fecundity and opening of *D. insulare* (from the above study).

Nectar-collecting behaviour of D. insulare. ***Choice tests***. Stalks of three flowers of each species were inserted through holes in the lid of a 300 ml plastic container filled with sucrose solution (0.5 g/ml). The flower species were randomly arranged in a circle about 4.0 cm from the center of the cover. A second 300 ml container, with 1.5 cm diameter screened holes in the side, was put upside down on the first container and fastened with tape, creating a testing arena. We randomly arranged the arenas parallel to the white inflorescence light, 30 cm from the bulb. An unfed female *D. insulare* (1-d old) was released in the center of the testing arena through a hole in the upper container. Females were allowed to acclimatise for 2 h in the arena before observation. The nectar-collecting behaviour of *D. insulare* included the following; tried to get in or entered corolla tube, kicked sepal or petal, sucked or chewed at corolla base were observed. We also quantified the number of visits per flower species and the numbers of visits and time spent at the corollas. Behaviour were recorded using audio tape recorder for 30 min per observation session. These observation were repeated five times with new flowers and insects each time.

No-choice tests. Freshly emerged unfed adult *D. insulare* females were released into screen cages (30 x 30 x 20 cm, 30 *D. insulare* per cage) 1-d before the experiment to acclimatise them to the cage environment. We inserted stalks of flowers of each species into glass vials (3 flowers per vial) filled with sucrose solution. Six vials with flowers of a single species were put in the middle of each cage. Fifteen min after introduction of the flowers we recorded the numbers of individual *D. insulare* visiting the flowers using audio tape recorder in 30 sec. We then took out the flowers with vials. We introduced new flower species with vials in the another cage for the next observation. After the sixth cage we returned to the first cage and repeated this process five times (= five replicates per species).

Data analysis. Longevity and fecundity of *D. insulare*, ovipositional behaviour of *D. insulare* fed on different food sources, the number of visits and time spent per visit on flower, and comparisons of total visitors per 30 sec for each flower species were analyzed using 1-way ANOVA, and means were separated using the Fisher Protected Least Significant Difference (LSD) test. Relationships between longevity and fecundity and length and opening

Table 1. Longevity and fecundity (± S.E.) of *Diadegma insulare* females fed on various wildflowers as nectar sources during summer 1993[1]

Food Sources	June		July		August		September	
	L	F	L	F	L	F	L	F
B. vulgaris	14.5 ± 4.3b	104.3 ± 12.6b	*	*	*	*	*	*
E. cheiranthoides	2.5 ± 0.5ef	5.2 ± 0.7f	*	*	*	*	*	*
C. bursa - pastoris	8.5 ± 2.1c	33.5 ± 5.3d	*	*	*	*	*	*
T. arvense	4.2 ± 1.8d	8.5 ± 2.3e	*	*	*	*	*	*
L. campestre	3.2 ± 2.2de	3.8 ± 0.8f	*	*	*	*	*	*
C. leucanthemum	2.2 ± 0.5f	7.3 ± 1.4f	*	*	*	*	*	*
R. crispus	8.0 ± 2.1d	6.3 ± 0.5c	*	*	*	*	*	*
B. incana	*	*	10.2 ± 1.6c	68.5 ± 7.2d	11.5 ± 2.6d	75.8 ± 5.6e	*	*
B. kaber	*	*	19.2 ± 4.6a	130.5 ± 16.5a	23.2 ± 3.1a	160.3 ± 23.2a	*	*
D. carota	*	*	19.1 ± 3.2a	120.7 ± 14.2b	19.5 ± 2.1b	132.1 ± 7.8bc	*	*
S. arvensis - aphids	*	*	2.2 ± 1.3d	5.4 ± 1.2e	*	*	4.3 ± 1.3d	6.7 ± 0.5c
S. arvensi + aphids	*	*	*	*	*	*	12.1 ± 4.7c	65.4 ± 12.2b
C. album - aphids	*	*	1.4 ± 0.4d	0e	*	*	2.5 ± 0.3e	0c
C. album + aphids	*	*	*	*	*	*	13.6 ± 5.3b	74.3 ± 8.5b
Honey±water	19.4 ± 3.2a	128.2 ± 5.3a	18.2 ± 2.5ab	127.5 ± 7.4ab	19.3 ± 2.3b	140.4 ± 8.9b	22.2 ± 5.4a	135.4 ± 20.2a
No food	2.1 ± 0.4f	0g	2.0 ± 0.2d	0e	2.4 ± 0.8e	0f	2.2 ± 1.6e	0c

[1]Result in May was somewhat similar to the result of June, 1993; *, not tested or was not found in the field; L, longevity (day); F = fecundity (total number of *D. insulare* pupae formed of parasitized DBM larvae); Columns with different letters are significantly different (Fisher Protected LSD, $P < 0.05$)

diameter of flower corolla, ovipositional behaviour of *D. insulare* within 1 min of exposure to DBM larvae were analyzed using regresssion analysis.

Result and discussion

Longevity. Result in May which was somewhat similar to June is not presented here. In June, parasitoid longevity was significantly longer when fed on *B. vulgaris* than with other flowers, including *R. crispus* L. and *C. leucanthemum* L. *(Table 1)*. In July, *D. carota* and *B. kaber* had similar effects on the longevity of *D. insulare*, comparable to honey+water. Although *D. insulare* longevity on *B. incana* was shorter than on *B. kaber* it supported *D. insulare* better than *C. album* and *S. arvensis*. In August, longevity of *D. insulare* was higher when fed on *B. kaber* than on other food sources. In September, *C. album* and *S. arvensis* offered aditional food for *D. insulare.* These weeds haboured bean aphids, *Aphis fabae* (Scopli), which apparently provided honeydew for *D. insulare* to live longer on weed+aphids than on weeds-aphids. However, longevity of *D. insulare* fed on these weeds with aphids was significantly less than on honey+water indicating honeydew may not have certain sugars or essential amino acids or they may be present in insufficient quantity compared to floral nectar (Baker and Baker, 1983).

Fecundity. Fecundity of *D. insulare* in June was significantly higher when *B. vulgaris* flowers or honey+water was used as food, compared with other foods offered *(Table 1)*. In July, parasitoid feeding on *B. kaber*, *D. carota* or honey+water resulted in higher fecundity than on other food sources. Although *B. incana* was not as beneficial for *D. insulare* fecundity as *B. kaber*, it still offered better food than the non-brassicas wildflowers, *S. arvensis* and *C. album*. In August, fecundity of of *D. insulare* was significantly higher when fed on *B. kaber* than on other food sources. Longevity and fecundity of *D. insulare* fed on *B. kaber* were significantly higer than when fed with honey+water in August, suggesting that *B. kaber* is an excellent food for *D. insulare*. Fecundity of *D. insulare* fed on *C. album* or *S. arvensis* with aphids was significantly higher than these weeds-aphids

Oviposition behaviour. The frequency of ovipositional behaviour made by *D. insulare*, within 1 min of exposure to the larvae, was signifcantly higher when they were fed on *D. carota*, *B. kaber*, or honey+water than when fed on *B. napus*, *B. incana*, *C. bursa-pastors* or *T. arvense*. However, result showed otherwise for the other two time intervals (1–5 or 6–20 min). Longevity and fecundity of *D. insulare*, which we consider as indices of food quality, strongly correlated with the frequency of individual parasitoids initiating oviposition behaviour within 1 min of exposure to the host [$r = 0.91$, $F = 255.7$, $P < 0.05$ (longevity); $r = 0.87$, $F = 14.8$, $P < 0.05$ (fecundity)].

Relationship of flower characters to D. insulare longevity and fecundity. Regression analysis indicated that 14.4% and 59.9% of variation in the longevity of *D. insulare* could be explained by the corolla length and opening diameter, respectively. There was a significant positive correlation between *D. insulare* longevity and corolla openinga and length even though a negative correlation was expected with corolla length, if a narrow corolla limited access to nectar by *D. insulare* (*Fig. 1a* and *b*). For *D. carota* its extremely short corolla length plus widely open corolla opening did not influence longevity. *B. kaber* petals are separated down to the base of the corolla providing easy access of the parasitoid to the nectaries, in spite of its length. There was no significant relationship between the corolla length and the fecundity of *D. insulare (Fig. 2a)*. However, corolla opening explained 75% of the variation in *D. insulare* fecundity *(Fig. 2b)*. There are also other factors affecting access to nectar besides corolla length and opening. For example, thickness of the petals and sepals at the base of the corolla and sepals attached at the base, covering the bottom half of the corolla may also be important.

Nectar collecting behaviour of *D. insulare*. In choice tests, we observed five distinct nectar-collecting behaviours of *D. insulare (Table 2)*. D. insulare tried to get in the corolla through the corolla opening of all flowers species. However, they only successful in entering the corolla of *B. incana*, *T. arvense*, *C. bursa-pastoris* and *D. carota*. Kicking the soft, separated sepal or petal, was observed on *B. incana*, *E.*

Table 2. Nectar-collecting behaviour of *Diadegma insulare* females observed in choice test with various flowers

Behaviour	Flowers							
	B. vulgaris	*B. napus*	*B. kaber*	*E. cheiranthoides*	*T. arvense*	*C. bursa-pastoris*	*D. carota*	*B. incana*
Tried to get in corolla tube	+	+	+	+	+	+	+	+
Entered corolla tube	–	–	–	–	+	+	+	+
Kicked sepal or petal	–	–	–	+	+	+	–	+
Sucked or chewed at corolla base	+	+	+	–	–	–	–	–
Circled at the corolla base	+	+	+	+	+	+	–	+

+, yes; –, no

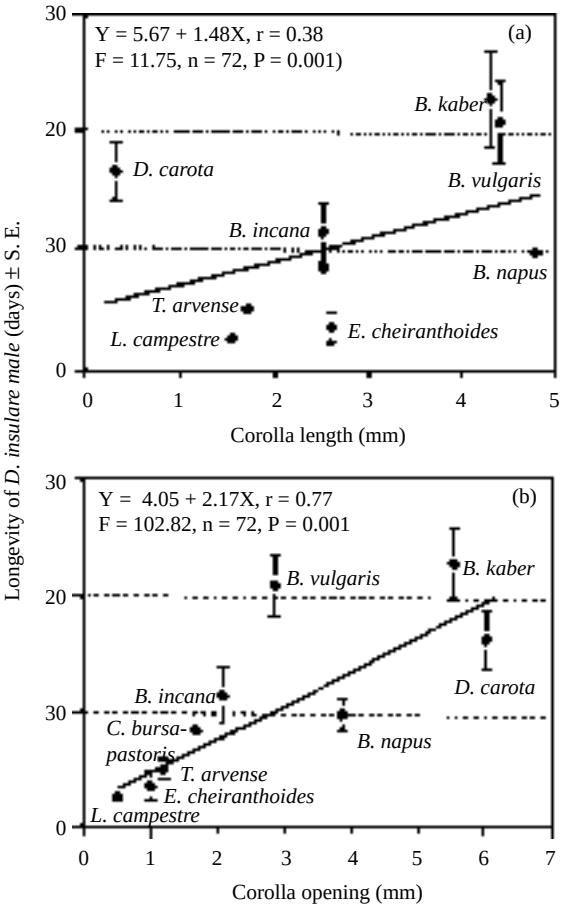


Figure 1. Longevity of D. insulare females in relation to corolla length (a) and opening (b) of wildflowers

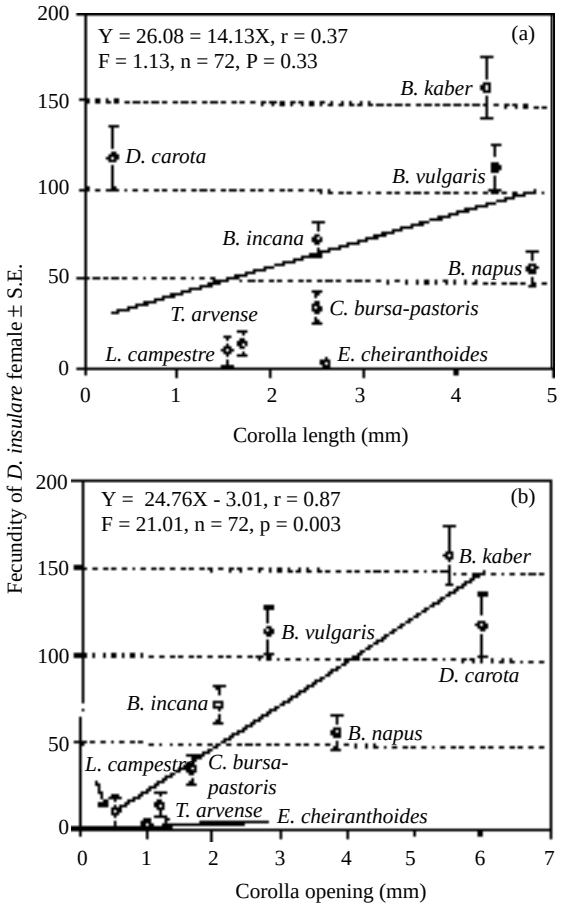


Figure 2. The relationship of the fecundity of D. insulare females to the corolla length (a) and opening (b) of wildflowers

cheiranthoides, *T. arvense* or *C. bursa-pastoris*. *D. insulare* did not try to entered the corolla tubes of *B. kaber* flowers because the corolla base has a wide separation between the sepals or petals, and between the sepals and petals; to reach the nectar at the base of the corolla, *D. insulare* could easily entered from the side. In contrast, sepals and petals of *E. cheiranthoides* are attached to form a corolla tube, and given the narrow corolla opening *D. insulare* could not entered the tube or reach the nectar. In contrast, *D. insulare* easily entered the wide, shallow corolla of *D. carota*. *D. insulare* circled the corolla bases of all flower species offered, indicating a high affinity to get close to the actual food source. *D. insulare* appeared to suck or chew at the corolla base of *B. vulgaris* and *B. napus* flowers and these were subsequently found to have holes that probably released the floral nectar. Apparently, *D. insulare* used its mandibles to make the holes to reach the nectar. Theses results indicate that there is behavioural flexibility of *D. insulare* in collecting floral nectar. Most of these behaviours ware reported for bumble bees (Guiterman, 1959) and not for parasitoids, especially the ichneumonids (Jervis *et al*., 1993). Therefore, this is the first report that an ichneumonid can behave like bumblebee in trying to reach the floral nectar sources. *D. insulare* made significantly more visits to *B. vulgaris* than to other flower species *(Fig. 3a)*. They spent longer times per visit on *B. napus*, *B. vulgaris*, *B. kaber* and *D. carota* than on other flower species *(Fig. 3b)*. This suggest that visiting these flowers is more rewarding. The numbers of visit and times spent at the corolla bases were significantly higher on *B. vulgaris*, *B. napus* and *B. kaber* than on the other flower species *(Fig. 3a* and *b)*. *D. insulare* did not visit the base of *D. carota* corolla because its corolla tube is extremely short and widely open.

In no-choice tests, there were significantly more visitors to *B. kaber*, *B. vulgaris* and *D. carota* than to other flowers *(Fig. 4)*. There were fewer visitors to *E. cheiranthoides* flowers than to any other flower species, reflecting the poor quality or quantity of its nectar. Results may be different in nature as the diversity and abundance of wildflowers vary with habitat or landscape. In the field, like our no-choice tests, some flower types are visited more frequently or have more visitors than would be expected, based on their respective abundance.

In choice tests, there was no significant difference in the number of visits to *E. cheiranthoides*, *B napus* (yellow) or *D. carota* (white) flowers made by *D. insulare (Fig. 3a)*. Whilst in no-choice tests the numbers of visitors on *D. carota* were as high as on *B. vulgaris* (yellow) but signifiantly higher than on *B. incana* (white) *(Fig. 4)*. Therefore, colour appeared

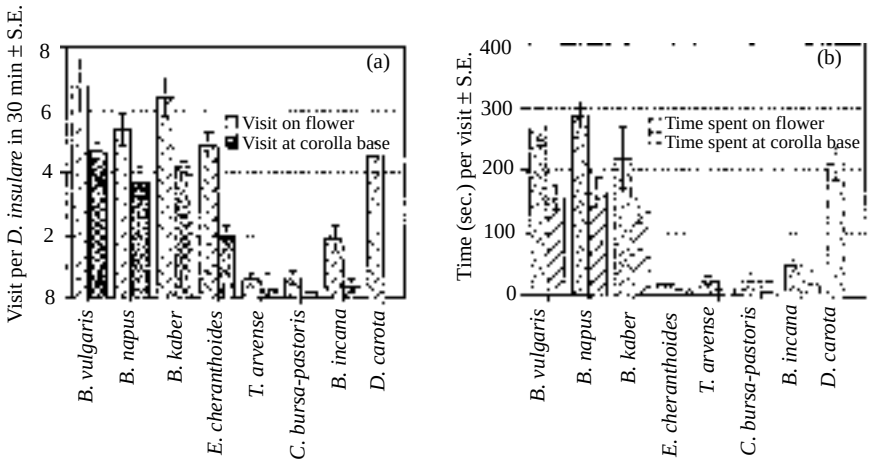


Figure 3. Number of visit (a) and time spent per visit (b) on flower or at the corolla base made by D. insulare females in 30 minutes of choice experiments

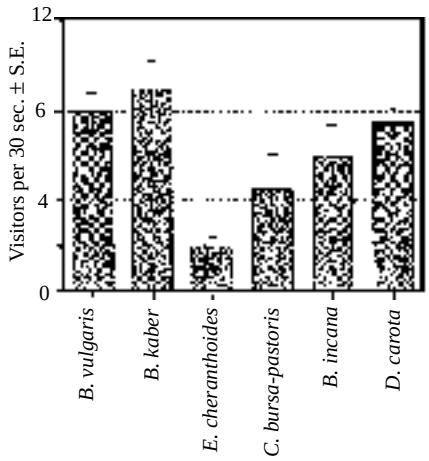


Figure 4. Numbers of D. insulare females (visitors) per flower species per 30 sec. in no-choice test experiments

not to be a factor determining a nectar-collecting behaviour of *D. insulare*.

Although most Ichneumonids lacking elongated mouth part that excluded them from using the nectar of many plants especially of Asteraceae, Leguminosae and Convulaceae (Jervis *et al*., 1993), results of our study indicated that there was a behavioural flexibility of *D. insulare* in collecting nectar of flowers with different morphological characters. It is possible that *D. insulare* longevity and fecundity are determined by its behavioural flexibility in collecting nectar, and the availability and acessibility of nectar sources in and around the field. The width of the corolla has a strong effect on both longevity and fecundity, but it did not explain all the observed variation between wildflower species as food sources. Nectar quality and extrafloral nectar are probably other important factors determining the longevity and fecundity of *D. insulare* (Baker and Baker, 1983) but we did not measure them. *C. album* and *S. arvensis* that did not have accessible nectar could indirectly provide food sources by harboring aphids that produce honeydew for the parasitoid. Flowers with accessible nectar might help increase parasitism of DBM. *B. vulgaris*, *B. kaber*, and *D. carota*, which are abundant in weedy areas and idle fields in Michigan during early and middle-to-late season, respectively, could influence effectiveness of *D. insulare* as a biocontrol agent of DBM. The distribution of these weeds which flower has accessible nectar should be manipulated in cabbage cropping systems to favour *D. insulare*. Design of crop management systems including management of natural enemy food sources (for example, planting *B. kaber*, *B. vulgaris* or *D. carota* in the cabbage field ecosystem) will become more important as we try to integrate biological control with production of high value vegetable crops.

Acknowledgement

Special thanks to James Miller, James Kells, Walter Pett and Beth Bishop (Michigan State University) for their valuable assistance and critical comments. We are also indepted to S. Stephenson (Michigan State University) for weed identification. Research was supported by the Michigan Agriculture Experiment Station and the Michigan Vegetable Council.

References

Baker, H. G., and Baker, I. 1983. A brief historical review of the chemistry of the floral nectar. In Bentley, B. and Elias, T. [eds.], *The biology of nectar*. Columbia University Press, New York, pp. 126–152.

Cowgill, S.E., Wratten, S.D,, and Scotherton, N.W. 1993. The selective use of floral resources by the hoverfly *Episyrphus balteatus* (Diptera: Syrphidae) on farmland. *Annual Applied Bioliology*. ***122:*** 223–231.

Fitton, M., and Walker, A. 1992. Hymenopterous parasitoids associated with diamondback moth: the taxonomic dilemma, . In Talekar, N.S. [ed.*], Diamondback moth and other crucifer pests: Proceedings, 2nd International Workshop*, 10–14 December 1990, Tainan, Taiwan. Asian Vegetable Research and Development Center, Shanhua, Taiwan, pp. 225–237.

Guiterman,A. 1959. Bumble bees and flowers. pp, 100–122. In. Free, J. B, Butler, C.G., and Yarrow, I.H.H. (eds.), *Bumblebees*. The MacMillan Company, New York.

Harcourt, D.G. 1986. Population dynamic of the diamondback moth in southern Ontario, pp. In Talekar, N.S. and Griggs, T.D. [eds.], *Diamondback moth moth*

management. Proceedings, 1st. International Workshop, 11–15 March 1985, Tainan, Taiwan. Asian Vegetable Research and Development Center, Shanhua, Taiwan, pp. 1–15.

Idris, A.B. 1995. Ecology and behaviour of *Diadegma insulare* (Cresson), a biological control agent of diamondback moth, *Plutella xylostella* (L.). *Ph.D. dissertation*, Michigan State University, East Lansing.

Idris, A.B. and Grafius, E. 1993a. Field studies on the effect of pesticides on the diamondback moth (Lepidoptera: Plutellidae) and parasitism by *Diadegma insulare* (Hymenoptera: Ichneumonidae). *Journal of Economic Entomology*. ***86:*** 1 196–1 202.

Idris, A.B. and Grafius, E. 1993b. Pesticides affect immature stages of *Diadegma insulare* (Hymenoptera: Ichneumonidae) and its host, the diamondback moth (Lepidoptera: Plutellidae). *Journal of Economic Entomology*. ***86:*** 1 203–1 212.

Jervis, M.A., Kidd, N.A.C., and Fitton, M.G. 1993. Flower-visiting by hymenopteran parasitoids. *Journal of Natural History*. ***27:*** 67–105.

Keven, P.G., Baker, H.G. 1984. Insects and flowers. pp. 607–631. In Huffaker, C. B. Rabb, R.L. 9eds.), *Ecological Entomology*. John Wiley and Son, New York.

Leius, K. 1967. Food sources and preferences of adults of a parasites, *Scambus buolianae* (Hymenoptera: Ichneumonidae), and their consequences. *Canadian Entomologist*. ***99:*** 865–871.

Lim, G.S., Sivapragasam, A. and Ruwaida, M. 1986. Impact assessment of *Apantales plutellae* on diamondback moth using insecticide-check method.. In Talekar, N. S. and Griggs T. D. [eds.], *Diamondback moth moth management. Proceedings, 1st International Workshop,* 11–15 March 1985, Tainan, Taiwan. Asian Vegetable Research and Development Center, Shanhua, Taiwan, pp. 195–204.

Syme, P.D. 1975. The effects of flowers on the lonngevity and fecundity of two native parasites of the European pine shoot moth in Ontario. *Environmental Entomology*. ***4:*** 337–346.

Van Emden, H.F. 1963. An observation on the effect of flowers on the activity of parasitic Hymenoptera. *Entomol. Mon. Mag*. ***98:*** 265–270.

Zhao, J.Z., Ayers, G.S., Grafius, E.J., and Stehr, F.W. 1992. Effects of neighboring nectar-producing plants on populations of pest Lepidoptera and their parasitoids in broccoli plantings. *Great Lakes Entomologist*. ***25:*** 253–258.

Characteristics of parasitism of diamondback moth by *Oomyzus sokolowksii* (Hymenoptera: Eulophidae)

N. S. Talekar

Asian Vegetable Research and Development Center, Shanhua, Tainan 741, Taiwan, ROC

Abstract

Laboratory, greenhouse and field studies were conducted on *Oomyzus sokolowskii* Kurdjumov, a parasite of diamondback moth, *Plutella xylostella* (L.), to judge its suitability for introduction in the field to control the plutellid. *Oomyzus sokolowskii* preferred the third and fourth instar diamondback moth larvae over fresh pupae for parasitization. It is thus a larval parasite. Within the range of 10 °C to 35 °C, the higher the temperature the higher was the parasitism rate. High parasitism at temperatures of 30 °C and 35 °C indicates that this insect is suitable for introduction in the tropical lowlands. In a no-choice test where only fresh pupae of *Cotesia plutellae* Kurdjumov (another potentially competing larval parasite of diamondback moth) were offered, *O. sokolowskii* failed to parasitize the pupae. In a choice test where the fourth instar diamondback moth larvae and fresh *C. plutellae* pupae were offered, *O. sokolowskii* parasitized only diamondback moth larvae. This parasite, therefore, is not a hyperparasite of diamondback moth. When *C. plutellae*-oviposited diamondback moth larvae were offered at intervals for parasitism by *O. sokolowskii*, it parasitized only freshly oviposited host larvae. The longer the period that elapsed after *C. plutellae* oviposition of diamondback moth larvae, the lesser was the parasitism of these larvae by *O. sokolowskii*. In a field cage study, as the diamondback moth population increased, the parasitism of the pest by the eulophid increased, parasitism by *C. plutellae*, however, decreased. Host-plant (cabbage) age did not affect the parasitism of diamondback moth larvae by *O. sokolowskii*; in both seedlings and mature plants the level of parasitism of the plutellid larvae was comparable. Most organic insecticides tested were toxic to both pupae and adults of *O. sokolowskii* but *Bacillus thuringiensis* was not toxic. Introduction of *O. sokolowskii* in a large field cage erected over a cabbage field reduced the infestation of cabbage by diamondback moth and doubled the yield of cabbage over the control plot where no parasite was used.

Key words: *Oomyzus sokolowskii*, diamondback moth, parasitism, temperature effect, hyperparasitism, parasite competition

Introduction

In tropical to subtropical Asia, major cruciferous vegetables are grown in two distinct ecological zones: cool highlands and hot lowlands. In both areas, diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is a serious pest. The climate, especially temperatures, in the highlands resembles that of temperate areas. In the lowlands, high temperatures reaching and at times exceeding 35 °C are common. In Europe, where the diamondback moth originated, the pest is kept under control by a plethora of temperate-climate natural enemies, mainly parasites (Mustata, 1992). Introduction of some of these temperate-climate parasites, especially *Diadegma semiclausum* Hellen, and to some extent *Diadromus collaris* Gravenhorst, in countries in Asia, has resulted in their establishment in highlands resulting in considerable reduction in the severity of diamondback moth damage (Talekar, 1996a). In lowlands, however, none of these parasites is effective due to their sensitivity to high tempeatures. *Cotesia plutellae* Kurdjumov, which is tolerant to the high temperatures commonly found in the tropical lowlands (Talekar and Yang, 1991), occurs naturally in Taiwan (Wu, 1968), Philippines (Velasco, 1983), Malaysia (Lim and Ko, 1975) and Thailand (Keinmeesuke, 1992). Although this braconid is an important mortality factor in the lowlands, its effectiveness in controlling diamondback moth has not been consistent. Therefore, another parasite, *Oomyzus sokolowskii* Kurdjumov (Hymenoptera: Eulophidae), which has given very effective control of the plutellid pest in Cape Verde Island (Lima and van Harten, 1985; Carl, 1992), was imported from that tropical West African country via the International Institute of Biological Control for introduction in lowlands of Asia. Several laboratory, greenhouse, and field experiments have been conducted to study characteristics of its parasitism to judge the suitability of this parasite for introduction in tropical to subtropical areas of Asia.

Preference of O. sokolowskii for various immature stages of diamondback moth

Several reports indicate that *O. sokolowskii* is a pupal parasite (Bennet and Yaseen, 1972; Wakisaka *et al.* 1991; Chelliah and Srinivasan, 1986). In our first study we compared the parasitism of diamondback moth pupae with that of third and fourth instar larvae.

Ten third and fourth instar larvae and freshly formed diamondback moth pupae were placed on a cabbage leaf in a 1.5-liter plastic container. Thirty 5-day-old *O. sokolowskii* adults were then introduced

in the container for oviposition. We then removed the host larvae and maintained them until pupation. The numbers of larvae pupating into diamondback moth or parasite pupae were recorded. We maintained the plutellid pupae that were also exposed to *O. sokolowskii* for oviposition and recorded the number of pupae that produced the plutellid adults and the number that produced parasite adults. This experiment was conducted three times, each time with three replicates.

In the second experiment, 20 first, second, third, and fourth instar larvae and 20 freshly formed pupae of diamondback moth were placed on large cabbage leaves inside each of four 1.5-liter round jars. Two hundred *O. sokolowskii* adults were then introduced in each jar and allowed 24 hours for oviposition. The number of pupae that developed into plutellid adults and the number that produced parasite adults were recorded. Host larvae were reared until pupation and the number of those pupating into diamondback moth or *O. sokolowskii pupae* was recorded.

The rates of parasitism of immature stages from the above two experiments were analyzed by ANOVA. Mean percentage parasitism was compared by the test of Least Significant Difference (LSD).

Oomyzus sokolowskii parasitized the third and fourth instar diamondback moth larvae (average 70% and 61%, respectively) but failed to parasitize pupae *(Table 1)*. It is thus a larval parasite. *Cotesia plutellae,* another larval parasite of diamondback moth with similar ecological requirements as *O. sokolowskii*, parasitizes second, third, and fourth instar plutellid larvae with preference for the second instar (Talekar and Yang 1991).

In most of southeast Asia where *C. plutellae* is established, the introduction of *O. sokolowskii* might result in competition between the two parasites. This led us to study the preference of *O. sokolowskii* adults to parasitize various instars of diamondback moth. Results *(Table 2)* indicate that *O. sokolowskii* prefers third and fourth instars over first and second instars. The preference of *O. sokolowskii* for third and fourth instars and that of *C. plutellae* for second instar could reduce competition between these two larval parasites, assuring survival of both. This could contribute to the biological control of diamondback moth in lowland areas of Asia where this plutellid pest is especially serious. However, the reduction of competition will depend on the interval between parasitism of second instar host larvae by *C. plutellae* and molting into third instar when chances of its being attacked by *O. sokolowskii* increases. The longer the interval, the better are the chances of survival of *C. plutellae* (see later discussion).

Effect of temperature on parasitism

Three-to five-day-old *O. sokolowskii* adults and third and fourth instar diamondback moth larvae were separately maintained at various temperatures: 10, 15, 20, 25, 30, and 35 °C for 6 hours to condition both host and parasite to these temperatures. Fifty

Table 1. Preference of *O. sokolowskii* for parasitism of various immature stages of diamondback moth

Test	Parasitism (%)		
	3rd instar larvae	4th instar larvae	Pupae
First	66.6±20.8	52.2±19.5	0
Second	68.9±20.1	67.5±27.0	0
Third	75.2± 4.5	81.8± 5.1	0
Mean	70.2± 4.5	67.2±14.8	0

Ten larvae or fresh pupae of diamondback moth were exposed to 30 *O. sokolowskii* adults for oviposition.

Table 2. Preference of *O. sokolowskii* for parasitism of various instars and pupae of diamondback moth

Instar	Parasitism (%)[a]
First	25.00 + 14.14
Second	41.25 + 17.01
Third	73.75 + 16.01
Fourth	76.25 + 14.36
Pupae	0
LSD[b]	20. 80

[a]Parasitism data are means + standard deviation of 4 replicates

[b]LSD = Least significant difference

O. sokolowskii adults were then introduced in a 15-cm-diameter, 30-cm-long acrylic cylinder containing 50 plutellid larvae feeding on a cabbage leaf. Both ends of the cylinder were covered with a single layer of fine muslin cloth. Four such cylinders containing parasite adults and host larvae were maintained at 10, 15, 20, 25, 30 or 35 °C for 24 hours. During this period, *O. sokolowskii* adults laid eggs in diamondback moth larvae. After 24 hours, the pest larvae were maintained at 25 ± 2 °C and reared until pupation. At pupation the number of larvae developing into diamondback moth pupae and parasite pupae were recorded and percent parasitism was calculated. A simple linear regression correlation between temperature and percentage parasitism was calculated (Little and Hills, 1975).

As the temperature increased from 10 °C to 35 °C, parasitism of diamondback moth by *O. sokolowskii* increased significantly ($r = 0.987$, $p = 0.01$, *Figure 1)*. High parasitism at temperatures of 30 °C and 35 °C indicates that this parasite might be suitable for the tropical lowlands. Parasite mortality ranged from 4.5% to 14.6% and was not related to temperature. *Oomyzus sokolowskii* is now being introduced in crucifer-growing lowland areas of Taiwan, Thailand, and Malaysia.

Hyperparasitism study

Because environmental conditions, especially temperature, for survival and multiplication of *C. plutellae* and *O. sokolowskii* are similar, and because *O. sokolowskii* is a minute insect, it has often been postulated – but never documented – that *O. sokolowskii* could be a hyperparasite of diamondback moth.

Both no-choice and choice tests were used to study the preference of *O. sokolowskii* for oviposition in

diamondback moth larvae or *C. plutellae* pupae. In the no-choice test, 50 fresh *C. plutellae* pupae were placed on a cabbage leaf in each of eight acrylic containers. Twenty two-day-old *O. sokolowskii* adults were then introduced for oviposition in each of four containers. The remaining four containers were maintained as control. The host and parasite insects were maintained together at 26 ± 2 °C for 48 hours. *Oomyzus sokolowskii* adults were then removed and *C. plutellae* pupae transferred individually into glass vials to observe pupation and emergence of *C. plutellae* or *O. sokolowskii* adults.

In the choice test, 50 fourth instar diamondback moth larvae and 50 fresh *C. plutellae* pupae maintained on a cabbage leaf were placed in each of four acrylic containers. Twenty two-day-old *O. sokolowskii* adults were then introduced inside each of these containers and allowed to lay eggs for 48 hours. The plutellid larvae and *C. plutellae pupae* were then maintained separately at 25 ± 2 °C. The number of diamondback moth larvae producing parasite pupae and the number developing into diamondback moth pupae were recorded. *Cotesia plutellae* pupae were observed and the number of those that showed parasitism by *O. sokolowskii* was recorded.

In both choice and no-choice tests, *O. sokolowskii* failed to parasitize *C. plutellae* pupae *(Table 3)*. In the choice test *O. sokolowskii* parasitized the fourth instar diamondback moth larvae but not *C. plutellae* pupae. It was not possible to expose larvae of *C. plutellae* and diamondback moth for *O. sokolowskii* oviposition simultaneously for fair comparison because *C. plutellae* larvae develop inside diamondback moth larvae. Instead we used freshly formed *C. plutellae* pupae. The fact that in the no-choice test *O. sokolowskii* did not parasitize *C. plutellae* shows that *O. sokolowski*i is not a parasite of *C. plutellae* and thus is not a hyperparasite of diamondback moth.

It is possible that *O. sokolowskii* parasitizes other diamondback moth parasites such as *D. semiclausum* or *D. collaris*. However, *D. semiclausu*m is a cool climate parasite (Talekar and Yang, 1991) and *D. collaris*, which also prefers cool climate, is a pupal parasite. These factors rule out *O. sokolowskii* being a hyperparasite of diamondback moth. This finding allows the introduction of this parasite in all lowland areas of southeast Asia where *C. plutellae* also parasitizes diamondback moth.

Competition for parasitism between C. plutellae and O. sokolowskii

Since *C. plutellae* and *O. sokolowskii* share similar ecological niches, and they both attack diamondback moth larvae, it is possible that these insects would compete for host larvae and that the less competitive of the two species might not survive. Therefore, we performed an experiment to determine competition between the two parasites. Because *C. plutellae* can infest all four instars of diamondback moth (Velasco, 1983; Talekar and Yang, 1991) and because it is already established in most countries of Asia, we exposed the host larvae first to *C. plutellae* and then to *O. sokolowskii*.

Twenty fourth instar diamondback moth larvae were individually confined with a gravid *C. plutellae* female in a test tube. After oviposition, each plutellid larva was observed under the microscope to make sure that *C. plutellae* had indeed laid eggs in each of them. The parasite eggs could be seen through larval cuticle. At 0, 24, 48, 72, ans 96 hours after oviposition, the *C. plutellae*-oviposited larvae were individually exposed to oviposition by *O. sokolowskii*. We recorded the number of diamondback moth larvae pupating into *C. plutellae* or *O. sokolowskii* pupae. We also recorded the mortality of diamondback moth larvae and parasite pupae.

When *C. plutellae*-oviposited diamondback moth larvae were immediately exposed to parasitization by *O. sokolowskii*, most of the diamondback moth larvae were oviposited by the latter parasite and yielded *O. sokolowskii pupae (Figure 2)*. Even 24 hours after oviposition by *C. plutellae*, practically 50% of the plutellid larvae were successfully parasitized by *O. sokolowskii*. However, after 48 hours, practically all *C. plutellae*-oviposited diamondback moth became *C. plutellae* adults. It is possible that at 48 hours and later, *C. plutellae* embryos, which are in an advanced stage of development, kill *O. sokolowskii* eggs as they do *D. semiclausum* eggs (Yang *et al.*, 1994). This implies that *O. sokolowskii* could become the dominant parasite when introduced in an area where *C. plutellae* is already established. The latest information from Cape Verde Island indicates this to be the case (Carl, 1992). However, the fact that *C. plutellae* can parasitize earlier instars of diamondback moth larvae should enable the parasites to co-exist, as has been shown in

Table 3. Parasitism of *P. xylostella* or *C. plutellae* by *O. sokolowskii* in choice and no-choice test

Test condition	Parasitism (%)		Adults emerged (%)		Mortality (%)	
	P. xylostella	*C. plutellae*	*P. xylostella*	*C. plutellae*	*P. xylostella*	*C. plutellae*
No choice[(a)]	–	0	–	98.50 ± 1.91	–	1.50 ± 1.91
Choice[(b)]	30.90 ± 1.39	0	58.31 ± 8.08	97.50 ± 1.00	10.79 ± 7.04	2.50 ± 1.00

Data are means ± standard deviation of 4 replicates.
[(a)]Only *C. plutellae* pupae were offered for parasitism by *O. sokolowskii*
[(b)]Both 4th instar *P. xylostella* larvae and *C. plutellae* pupae were offered for parasitism by *O. sokolowskii*.

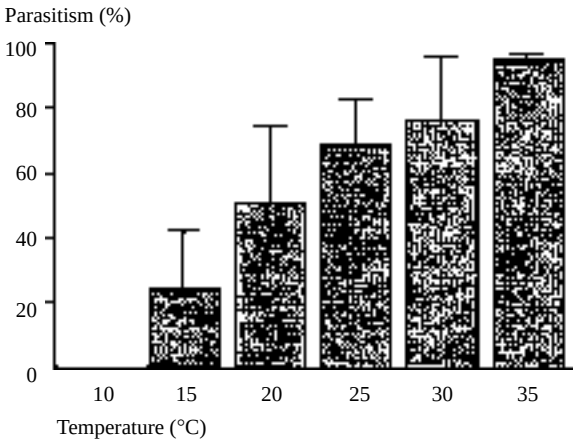


Figure 1. Parasitism of diamondback moth larvae by O. sokolowskii at various temperatures

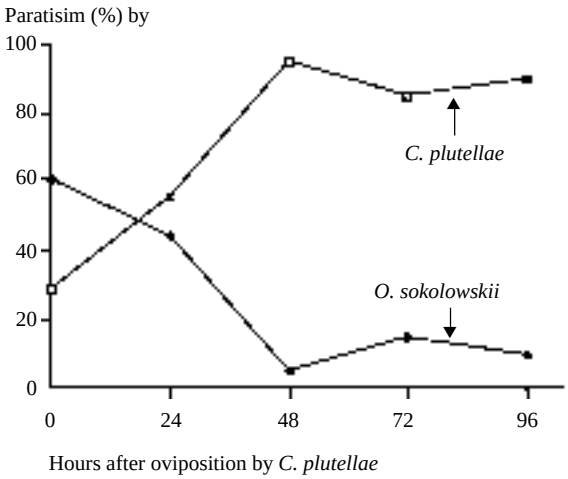


Figure 2. Effect of time elapsed after C. plutellae oviposition in diamondback moth larvae on the success of parasitism of the same larvae by O. sokolowskii. (Each point is the average of 20 larvae exposed to parasitism).

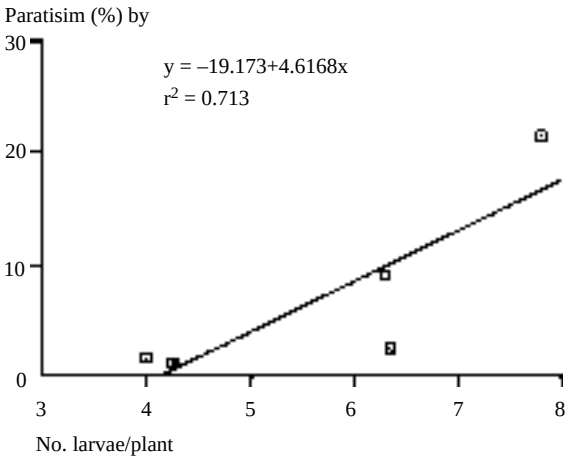


Figure 3. Relationship of population of diamondback moth larvae per plant and parasitism by O. sokolowskii

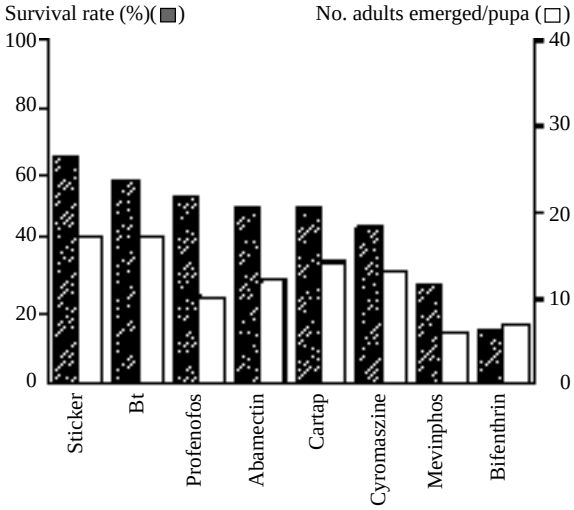


Figure 4. Effect of various insecticides on the survival of pupae and emergence of O. sokolowskii adults

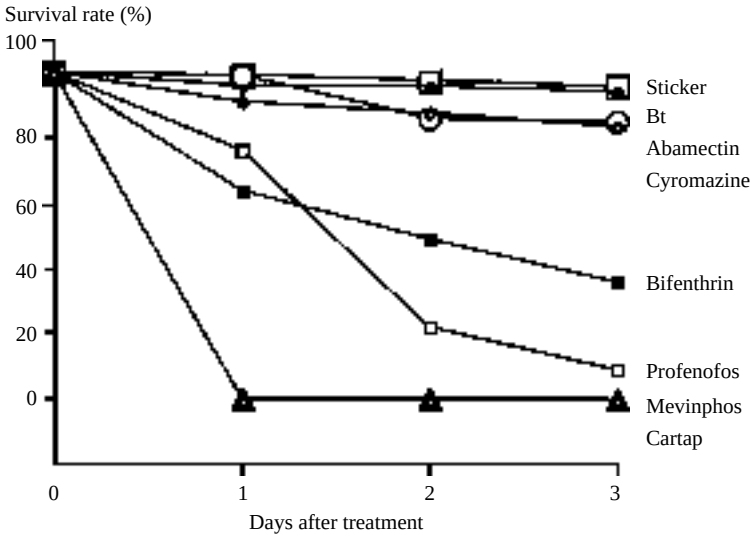


Figure 5. Effect of various insecticides on the survival of adults of O. sokolowskii

southern Japan, India, and the West Indies (Hirashima *et al.*, 1989, Chelliah and Srinivasan, 1986, Yaseen, 1978).

Relationship between diamondback moth larval population density and parasitism by O. sokolowskii

In this field experiment we studied the dependence of rate of *O. sokolowskii* parasitism on the density of diamondback moth larval population. In a 0.1-ha parcel of land planted to common cabbage we introduced 500 diamondback moth adults to initiate pest infestation. Two days later we introduced 1 000 *O. sokolowskii* adults in the same area. In addition, 500 parasite adults each were also introduced two, three, and four weeks later. Once a week we monitored the larval population of diamondback moth on cabbage plants and parasitism of the pest larvae by *O. sokolowskii* or *C. plutellae* which is already established in lowland areas of Taiwan. Diamondback moth population increased steadily from 10 days after the plutellid adult release up to harvest, from 2 to 22 per 10 plants. Parasitism of larvae by *O. sokolowskii* increased steadily from 1.3% to 21.9%. There was significant correlation (r = 0.844) between larval population density and the rate of parasitism *(Figure 3).* Parasitism by *C. plutellae* decreased from 48% to 3.1% during this period. In our earlier study we found similar decrease in parasitism of *C. plutellae* from the beginning towards the end of the season (AVRDC, 1992). Based on our present findings, it appears that introduction of *O. sokolowskii* will not compete but supplement the control of diamondback moth achieved by *C. plutellae*.

Effect of host-plant age on the parasitism of diamondback moth by O. sokolowskii

A field experiment was conducted to study the influence of host-plant (cabbage) age on the parasitism of diamondback moth larvae by *O. sokolowskii*. A parcel of land was rototilled and worked into 24, 1.5-m wide and 5.0-m long single bed plots. The whole area on four sides was confined by 2-m high fine-mesh net. The same mesh net was used to cover the top. Once every week for eight consecutive weeks we transplanted four-week-old cabbage seedlings in three randomly selected plots; one plot per replicate. Plants were maintained free of plutellid infestation. When the cabbage plants in the last transplanted plot were in the field for one week, we introduced 10 third-instar diamondback moth larvae per plant on each of 10 plants selected at random in each plot. The plants that received the pest larvae were marked.

Immediately after the introduction of diamondback moth we released 5 000 *O. sokolowskii* adults inside the cage for parasitism. At three and five days after parasite introduction, we collected 30 larvae from each plot and reared them in the laboratory until pupation. We recorded the number of *O. sokolowskii* and diamondback moth pupae that developed and computed the percent parasitism.

Parasitism varied between 18% and 54% in the first observation (three days) and 30 and 50% in the second observation (five days). There was no statistically significant relationship between plant age and parasitism. *Oomyzus sokolowskii* parasitism of diamondback moth is thus not affected by host-plant age. This parasite, therefore, can be introduced in the field for the biological control of the plutellid at any stage of plant growth.

Effect of various insecticides on the survival of O. sokolowskii

Our experience indicates that despite best efforts by researchers and extension authorities, some farmers continue to use insecticides in crucifer growing areas where parasites are newly established and diamondback moth is no longer a serious pest. Some of the insecticide use is for controlling insect pests other than diamondback moth. It is possible that some of the insecticides used could adversely affect diamondback moth parasites and thereby exacerbate diamondback moth problem. We conducted, therefore, a laboratory experiment to study toxicity of some commonly used insecticides to *O. sokolowskii*.

Commercial formulations of each of seven insecticides and one commonly used sticker were diluted as directed on the insecticide container for use in the field. Freshly formed pupae of *O. sokolowskii* were then dipped in insecticide solution for five seconds. Treated pupae were air-dried under a gentle flow of air. The dry pupae were placed at 28 ± 2 °C and emergence into *O. sokolowskii* adults was monitored. The number of pupae that failed to emerge were considered as dead. The total number of adults that emerged from surviving pupae were recorded.

In a simultaneous test with adults, the insecticide solutions were sprayed on fresh cabbage leaves. The treated leaves were placed in a 15-cm diameter acrylic cylinder. Twenty *O. sokolowskii* adults were released inside each cylinder and insect mortality was recorded at 72 hours after treatment.

The survival rate and the number of *O. sokolowskii* adults which emerged from the surviving pupae are shown in *Figure 4*. Bifenthrin was the most toxic and *Bacillus thuringiensis* were the least toxic insecticides. The order of toxicity was bifenthrin > mevinphos > cyromazine > cartap > abamectin > profenofos > *B. thuringiensis.* For adults, mevinphos and cartap were equally toxic and *B. thuringiensis* was the least toxic *(Figure 5).* The order of toxicity was mevinphos = cartap > profenofos > bifenthrin > abamectin = cyromazine > *B. thuringiensis.* The sticker was nontoxic to adults and its toxicity to pupae was lower than any products included in the test. *Bacillus thuringiensis* was the least toxic to both adults and pupae. This biological insecticide is frequently used to control diamondback moth. Abamectin, at present the most popular chemical for the control of diamondback moth, was relatively less toxic to both stages of *O. sokolowskii*. Cyromazine is frequently used for the control of leaf miner. This chemical is relatively safer to *O. sokolowskii* adults but not to

pupae. Mevinphos, an old broadspectum organophosphorus insecticide, is at times still used for controlling diamondback moth. This chemical is highly toxic to the parasite, as is bifenthrin, a synthetic pyrethroid.

Control of diamondback moth by O. sokolowskii

Prior to the introduction of *O. sokolowskii* for the control of diamondback moth in farmers fields, we conducted a study at AVRDC to investigate the potential of this parasite under field conditions. A parcel of land planted to common cabbage was divided into three 27 m by 13.5 m plots. Each area was subsequently confined on all four sides and the top by fine mesh nylon net to prevent movement of insects between the plots. In two plots we introduced 1 000 diamondback moth adults each. The third plot was maintained insect free. One week later, in one of the two plots where the pest was introduced, we released all *O. sokolowskii* adults emerging from 1 000 parasitized diamondback moth cocoons. Additional 500 parasite cocoons were each released at two, three, and four weeks later. We monitored the extent of diamondback moth parasitism throughout the season and determined yield and marketable cabbage heads at harvest.

In the parasite-released cage, parasitism of the plutellid larvae by *O. sokolowskii* ranged from 1.28% to 21.74%. Parasitism, which was very low, hovering around 2% early in the season, increased to 21.74% two weeks before harvest. Diamondback moth larval population, which remained low (around 5 larvae/plant) through most of the season increased to 22.4 larvae per plant a week before harvest. We are unable to determine the reason for this sudden increase in population. The rate of parasitism was obviously not adequate to prevent pest population build up. In the diamondback moth-only plot, the larval population was high and reached 36.9 larvae/plant a week before harvest. In this plot, cabbage heads, on an average, weighed 1.43 kg per head. The head weight increased to 1.53 kg in the plot where both diamondback moth and *O. sokolowskii* were released. In the control plot, where no diamondback moth nor parasite was released, each cabbage head weighed 1.62 kg. Only 26.1% of the heads were marketable in the diamondback moth-only plot; marketability increased to 44% in the parasite-released plot but it was below 76.1% marketability of the control plot where no insects were released. Cabbage yield was 6.84 t/ha in the only diamondback moth-plot; it increased to 12.81 t/ha due to parasite introduction but it was still below the 21.44 t/ha obtained in the control plot. Marketable heads, rather than head weight, contributed to increased yield. Introduction of *O. sokolowskii* to control diamondback moth increased head weight, marketable heads, and practically doubled the yield of cabbage. These data indicate the merits of introduction of *O. sokolowskii* to control diamondback moth to increase cabbage yield.

References

AVRDC. (1992). 1991 Progress Report. Asian Vegetable Research and Development Center, Shanhua, Taiwan, 410 pp.

Bennet, F. D. and Yaseen, M. (1972). Parasite introductions for the biological control of three insect pests in the *Lesser Antilles and British Honduras. PANS.* ***18:*** 468–474.

Carl, D. (1992). DBM control project in Togo, Benin and Cape Verde. IOBC global working group on biological control of *Plutella*, Newsletter 1991, 24 pp.

Chelliah, S. and Srinivisan, K. (1986) Bio-ecology and management of diamondback moth in India, In Diamondback moth management: Proceedings of the first international workshop (ed Talekar N. S. and Griggs, T. D.) pp. 63–76. Shanhua, Taiwan: Asian Vegetable Research and Development Center.

Hirashima, Y., Abe, M., Tadauchi, O., Konishi, K., and Maeto, K. (1989). The hymenopterous parasitoids of the diamondback moth, *Plutella xylostella* (Lepidoptera, Yponomeutidae) in *Japan. Esakia* ***28:*** 63–73.

Keinmeesuke, P. (1992). Integrated pest management of diamondback moth on crucifers in Thailand. In Collaborative Vegetable Research in Southeast Asia: Proceedings of the AVNET-I Final Workshop and AVNET-II Joint Planning Meeting, pp 335–343. Shanhua, Taiwan: Asian Vegetable Research and Development Center.

Lim, G. S. and Ko, W. W. (1975). *Apanteles plutellae* Kurdj., a newly recorded parasite of *Plutella xylostella* (L.) in Malaysia. *MARDI Research Bulletin* ***3:*** 94–95.

Lima, M. L. L. and Van Harten, A. (1985). Biological control of crop pests in Cape Verde. Current situation and future programme. Rev. *Invest. Agraria, Centro de Estudos Agrarios, A, No.* ***1:*** 3–12. (in French).

Little, T. M. and Hills, F. J. (1975). Statistical methods in agricultural research. DAVIS, University of California.

Mustata, G. (1992). Role of parasitoid complex in limiting the population of diamondback moth in Moldavia, Romania. In Diamondback moth and other crucifer pests: Proceedings of the second international workshop (ed. Talekar, N. S. and Griggs, T. D.) pp. 203–211. Shanhua, Taiwan: Asian Vegetable Research and Development Center.

Talekar, N. S. and Yang, J. C. (1991). Characteristic of parasitism of diamondback moth by two larval parasites. *Entomophaga. 36:* 95–104.

Talekar, N. S. (1996a). Role of biological control in IPM of diamondback moth in Southeast Asia. Paper presented at the Workshop on Biological Control as a Cornerstone of IPM for Sustainable Agriculture in Southeast Asia, Serdang, Malaysia (in Press).

Talekar, N. S. (1996b). Biological control of diamondback moth in Taiwan – a review. Plant Protection Bulletin (Taiwan), (in press)

Velasco, L. R. I. (1983). Field parasitism of *Apanteles plutellae* Kurdj (Braconidae: Hymenoptera) on the diamondback moth on cabbage. *Philippine Entomologist* ***6:*** 539–553.

Wakisaka, S., Tsukuda, R. and Nakasuji, F. (1991). Life tables of diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) and effects of rainfall temperature and host plants on survival and reproduction. *Japanese Journal of Applied Entomology and Zoology* ***35:*** 115–122.

Wu, K. C. (1968). Bionomic study of *Plutella maculipennis* Curtis. *Journal of Taiwan Agricultural Research* ***17:*** 51–63.

Yang, J. C., Chu, Y. I. and Talekar, N. S. (1994) . Studies on the characteristics of parasitism of *Plutella xylostella* (Lep.: *Plutellidae*) by a larval parasite *Diadegma semiclausum* (Hym.: Ichneumonidae). *Entomophaga* ***39:*** 397–406.

Yaseen, M. (1978). The establishment of two parasites of the diamondback moth, *Plutella xylostella* (Lepidoptera, Plutellidae) in *Trinidad, W. I. Entomophaga* ***23:*** 111–114.

Bacillus thuringiensis Berliner subspecies *kurstaki* in the management of diamondback moth in India

G. Shankar, S. Mallikarjunappa, U. Ganesha Bhat, G. N. Kendappa and M.S. Mithyantha

Rallis Research Centre, Peenya II Phase, Bangalore - 560 058, India.

Abstract

The diamondback moth (DBM), *Plutella xylostella* has been a serious pest of cruciferous vegetables in many parts of India. There are certain "hot spots" where even the synthetic pyrethroids are not providing adequate protection to cabbages and cauliflowers. The multilocational field experiments have indicated that the DBM populations in many cabbage tracts in India are highly susceptible to the biopesticide, *Bacillus thuringiensis* Berliner, subspecies *kurstaki* (Btk). The recent introduction of Btk formulations in the country is expected to change the current pest management scenario in cruciferous vegetables. The authors emphasise the need for judicious use of Btk formulations with other effective insecticides to avert development of resistance to the biopesticide.

Key words: *Bacillus thuringiensis* subsp. *kurstaki* (Btk); Diamondback moth (DBM), Biobit, acephate, insecticide - mixtures, resistance, LC50 values.

Introduction

Diamondback moth (DBM), *Plutella xylostella* L. (Lepidoptera : Yponomeutidae) is the most destructive pest of crucifers all over the world. In India, DBM infests crops like cabbage, cauliflower, radish, knolkhol, turnip, beetroot and mustard (Chand and Choudhary, 1977; Dube and Chand, 1977; Jayarathnam, 1977; Singh and Singh, 1982).

In India, cruciferous vegetables, perhaps, are the ones that receive maximum spray rounds with an average of about 15 in a crop season; but the rainy season cabbage in Karnataka (Bangalore district) receives more sprays at shorter intervals while in seed production areas in Bihar 20–25 sprays are inevitable. Besides the approved insecticides a number of other insecticides like acephate, cartap hydrochloride, dichlorvos, methomyl, profenofos etc., are also used for DBM control. As some of these provide encouraging results the same are used for successive sprays. Tank mixtures of insecticides are popular among the cabbage farmers and the doses used are arbitrary and the choice of insecticides has no scientific basis. The trap crop using Indian mustard (Srinivasan and Krishnamoorthy, 1992) was found to be very effective in considerably reducing the number of sprays but unfortunately this simple and effective technology could not be propagated across the country and the use of insecticides remains a widely accepted choice.

At this juncture, the recent introduction of the biological insecticide, *Bacillus thuringiensis* Berliner subsp. *kurstaki* (Btk) is a welcome move. However going by the reported resistance of DBM to Btk in other countries (Shelton, *et al.*, 1993; Syed, 1992 and Tabashnik *et al.*, 1990), it is essential to workout plans to delay such possible development of resistance in India.

This paper is based on our laboratory and field studies carried out with the following objectives:

a) evaluation of Biobit WP® (Btk) and other promising insecticides
b) comparative efficacy of available Btk formulations in the market
c) scope of insecticide mixtures in DBM management.

Materials and Methods

Two formulations of Btk Biobit WP - 16000 IU/mg and Biobit HPWP - 32,0000 IU/mg, supplied by Novo Nordisk, Denmark (currently being manufactured by Abbott Laboratories) and the other commercial products dichlorvos (Nuvan 76 EC - Hindustan Ciba-Geigy), profenofos (Curacron 50 EC - Hindustan Ciba-Geigy), cartap hydrochloride (Padan 50 SP - Coromandal Indag), carbosulfan (Marshal 25 E - FMC, Philadelphia), fenvalerate (Sumicidin 20 E), cypermethrin (Ralothrin 25 E), acephate (Asataf 75 SP), chlorpyriphos (Tafaban 20 E), endosulfan (Endotaf 35 E) and quinalphos (Quinaltaf 25 E) - all from Rallis India were used for laboratory and field evaluations in our studies. Besides these, a mixture of acephate 300 g a.i. + cartap 300 g a.i./kg, formulated as soluble powder, was also evaluated for the control of DBM. All the studies were carried out during the years 1991 and 1992.

Laboratory bioassays

The DBM population originally collected from a field in Malur (50 km from Bangalore) in August, 1993 was reared on mustard seedlings without exposure to any pesticide. It was further multiplied and maintained in our laboratory and used in the bioassays. The LC_{50} values were determined using eighty larvae (4 days old) per concentration and each replicated 4 times. The

cabbage leaf-discs treated with test insecticides were individually placed in Petri-dish and the larvae were allowed to feed for 72 h after which the mortality was recorded. A larva was considered dead if it did not respond on prodding.

Field evaluations

Field evaluations were carried out under different agro-climatic conditions in the major cabbage growing tracts of Karnataka (Bangalore), Bihar (Ranchi), Gujarat (Meshana) and Haryana (Sonipat) representing south, east, west and northern zones of the country, respectively. Majority of the trials were conducted during the winter season (September - November) in the years 1991 and 1992 except in Bangalore wherein the experiments were conducted during summer season while in Gujarat one trial was conducted in the winter season in the year 1991 and the other in May 1992. All the field experiments were in randomised block design with plot sizes ranging from $6m^2$ to $20m^2$ (average 12 m^2).

Table 1. Susceptibility of DBM to some insecticides and *Bacillus thuringiensis* subsp. *kurstaki*

Insecticide		LC_{50} ppm (A.I.)	Slope
I.	**Approved for DBM control**		
1.	Biobit HPWP[a]	23.39[b]	1.983
2.	Endosulfan	131.34	2.956
3.	Chlorpyriphos	202.03	3.121
4.	Fenvalerate	260.69	1.665
5.	Cypermethrin	572.08	2.531
6.	Quinalphos	851.35	1.130
II.	**Not yet approved for DBM**		
7.	Carbosulfan	23.14	2.679
8.	Profenofos	84.68	3.850
9.	Cartap	122.07	3.692
10.	Dichlorvos	162.46	2.798
11.	Acephate	1 166.79	1.564

[a]*Bacillus thuringiensis* with 32 000 IU/mg
[b]µg/ml as formulation

Each treatment was adequately replicated to generate sufficient data for statistical analysis. The plots were separated by 2 guard rows. Hand operated knapsack sprayer was used for delivering the test compounds and 4 to 5 spray rounds were imposed in different trials at weekly intervals. The observations were made at weekly intervals after each spray on the number of live larvae on 10 fixed plants per plot randomly chosen at the time of initiation of the experiment. The yield data were recorded by harvesting the marketable heads in Gujarat (1991) and Haryana (1992).

Results

Bioassays

Toxicity of some insecticides (LC_{50} values) that are 'approved' and 'yet to be approved' for DBM control are presented in *Table 1*. Amongst the approved insecticides Biobit (Btk) was highly toxic to DBM (23.39 ppm), while quinalphos was the least toxic (851.35 ppm A.I.) Among the 'yet to be approved insecticides' DBM was most susceptible to carbosulfan (23.14 ppm A.I.) while acephate was least effective with an LC_{50} of 1166.79 ppm (A.I.).

Field efficacy of Bacillus thuringiensis

The results of the field studies conducted during the years 1991 and 1992 with Btk (Biobit WP 16000 IU/mg) are presented in *Table 2* and *3*, respectively. It can be seen that Biobit WP at 1000 g/ha was the most effective treatment in many of the locations in both the years. At Meshana (1992) it performed well even at 500 g/ha. acephate (0.1% A.I.), cartap (0.05% A.I.) and carbosulfan (0.05% A.I.) were the other effective insecticides while quinalphos (0.05% A.I.) was the least effective.

Insecticide mixture

The acephate + cartap mixture used in two concentrations was consistently effective and

Table 2. Efficacy of different insecticides and *Bacillus thuringiensis* (Biobit) on the control of DBM (1991)

Products	Conc. % A.I.	Mean No. of larvae/10 plants A week after			Yield t/ha
		4 sprays Bangalore	3 sprays Meshana	4 sprays Ranchi	Meshana
1. Biobit WP	1 000	1.85 a	1.49 a	2.87 ab	38.15 ab
2. Biobit WP	1 500	2.12 a	1.41 a	2.35 a	40.50 a
3. Fenvalerate 20 E	0.02	4.53 c	–	–	–
4. Cartap hydrochloride 50 SP	0.05	2.51ab	–	2.42 a	–
5. Acephate 75 SP	0.10	3.03b	1.55 a	2.32 a	36.75 b
6. Carbosulfan 25 EC	0.025	–	1.49 a	3.10 b	39.25 a
7. Carbosulfan 25 EC	0.05	2.58ab	1.41 a	2.63 ab	39.12 a
8. Quinalphos 25 EC	0.05	–	–	3.59 c	–
9. Water spray	–	3.36	4.65 b	4.65 d	31.37 c
SEm ±		0.37	0.15	0.19	0.80
CD at 5%		1.15	0.48	0.58	2.43

a Data $\sqrt{x+1}$ are transformed values
b Data mean of 4 replicates at Meshana and 3 replicates in other locations
c Means in each vertical column followed by the same letter are not significantly different at 5% level.
d Dose of Biobit as formulation/ha

Table 3. Efficacy of different insecticides and *Bacillus thuringiensis* (Biobit) on the control of DBM (1992)

Products	Conc. % A.I.	Mean No. of larvae/10 plants A week after				Yield t/ha
		5 sprays Bangalore	3 sprays Meshana	3 sprays Sonipat	3 sprays Ranchi	Sonipat
1. Biobit WP	500	–	2.37a	–	–	–
2. Biobit WP	1000	2.91a	2.14a	2.62b	2.54ab	33.77a
3. Biobit WP	1500	2.59a	1.74a	1.98a	3.14ab	37.63a
4. Carbosulfan 25 EC	0.025	3.28a	2.37a	3.91d	3.00ab	32.39a
5. Carbosulfan 25 EC	0.05	3.96a	2.37a	3.59cd	3.64ab	34.58a
6. Acephate 30% + Cartap 30% SP	0.06	2.43a	–	2.96bc	1.00a	34.66
7. Acephate 30 % + Cartap 30% SP	0.12	2.13a	–	2.63b	1.00a	36.35a
8. Cartap 50 SP	0.05	2.84a	2.26a	3.99b	1.00a	33.10a
9. Acephate 75 SP	0.1	3.63a	2.44a	3.14bc	3.77ab	36.21a
10. Cypermethrin 25 EC	0.025	5.90b	–	–	–	–
11. Water spray	–	7.58c	5.40b	5.84e	9.63c	18.21b
SEm ±		0.62	0.32	0.21	0.50	1.86
CD at 5%		1.84	0.99	0.63	1.52	5.58

a Means are $\sqrt{x + 1}$ transformed values.
b Data mean of 3 replicates
c Means in each vertical column followed by the same letter are not significant at 5% level.
d Dose of Biobit as formulation/ha.

promising *(Table 3)*. The mixtures was found to be most effective in Bangalore and Ranchi locations while in Sonipat it was next to Biobit WP 1500 g/ha. However, the observed difference in bioefficacy did not reflect in the yield of marketable heads from Sonipat area.

Table 4. Toxicity of some commercial formulations of Btk to DBM

Btk	LC_{50}[b] (ppm)	Slope	S.E. of slope
1. Biobit HPWP[a]	23.39	1.98	0.848
2. Source 'B'	22.64	1.55	0.466
3. Source 'C'	207.72	5.02	1.368
4. Source 'D'	240.36	7.08	1.564

a Biobit HPWP potency 32,000 IU/mg
b LC_{50} value in ppm with regard to respective formulations.
c Other commercial formulations are indicated as source B, C and D.

Comparative efficacy of commercial formulations of Btk

A comparative efficacy study of Btk formulations from three different sources conducted in the year 1994 showed a wide difference in their LC_{50} values (22.64 to 240.36 ppm) as seen from the data in *Table 4*. In the field also this difference was apparent as the per cent reduction of DBM varied from 76.2 to 99.6 after 3 sprays *(Table 5)*.

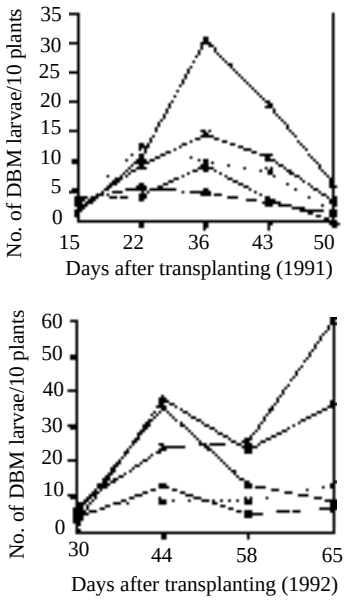
Resistance to pyrethroids

The results of the field studies conducted at Bangalore in the year 1991 showed that the pyrethroid fenvalerate recorded high population of DBM than that of control treatment after the 3rd spray *(Fig. 1)* and this trend continued even after the 5th application. A similar trend was observed with cypermethrin in the following year *(Fig. 1)*. Both Biobit 1000 g/ha and cypermethrin @ 0.025% A.I. showed very poor control of DBM after the 2nd spray. However in the subsequent 4th and 5th spray, Biobit was found to be good while cypermethrin continued to be ineffective.

Table 5. Field performance of commercial Btk formulations (Bangalore, February, 1994)

Btk[b]	Dosage g/ha	Mean larvae[a]/ plants				
		Before spray	3 days after I spray	% reduction	3 days after III spray	% reduction
Source 'A'	300	57.66	8.66	91.8	8.66	88.6
	600	84.66	1.66	98.4	14.00	81.5
Source 'B'	300	75.33	2.33	97.8	5.33	93.0
	600	76.66	2.00	98.1	0.33	99.6
Source 'C'	300	63.66	19.00	81.9	13.00	82.4
	600	63.00	8.66	91.8	18.00	76.2
Water spray	–	82.00	105	–	75.66	–

a Mean of 3 replicates
b Btk formulations from different manufacturers are indicated as source A, B, C.



Legends

- ■ Biobit WP 1000 g/ha
- ◆ Biobit WP 1500 g/ha
- ▲ Fenvelerate 0.02% a.i.
- ● Cypermethrin 0.025% a.i.
- -x- Acephate 0.1% a.i.
- x Water spray (control)

Figure 1. Field level tolerance of DBM to pyrethroids (Bangalore)

Discussion

The susceptibility of DBM to various insecticides was found to be similar both in the laboratory and field evaluations. Ironically the susceptibility was found to be more to the insecticides 'not yet approved' for use on DBM. Among the approved insecticides only Btk was highly effective both in the laboratory bioassay and in the field.

Interestingly, the performance of acephate was consistently good in the field but least effective in the bioassay (LC_{50} 1166.79 ppm A.I.). Being primarily a systemic insecticide, perhaps, it performed well in the field than in the bioassay where it could merely act as a contact poison. Secondly, the selective safety of acephate to *Cotesia plutellae* Kurdj (Hymenoptera : Braconidae) (Feng and Wang, 1984) perhaps, would have brought about an insecticide-parasitoid "synergism" to suppress the DBM population, in view of the ability of this parasitoid to parasitise DBM to the tune of 71.7 per cent (Yadav *et al.*, 1975).

Though a build-up of tolerance to synthetic pyrethroids was observed in Bangalore *(Fig.1)*, our other studies which are still in progress (at the time of preparation of this manuscript) in other parts of the country *viz.*, Hassan (Karnataka) and Nasik (Maharashtra) indicate a high to moderate susceptibility of DBM to these pyrethoids. This indicates the possibility of location -specific resistance which can be attributed to the abuse of synthetic pyrethorids in Bangalore.

Although the use of insecticide mixtures in the pest management schedules is controversial, the use of acephate + cartap combination in our trials was to find a balanced mixture of compounds with one of them having high efficacy against DBM and another one having a wide margin of safety to the parasitoid. Cartap was found to be highly toxic to DBM *(Table 1)* in our studies and it is also reported accordingly by Lim *et al* (1986). Acephate is reported to be least toxic to the parasitoid, *C. plutellae* (Feng and Wang, 1984) and hence the combination of these two products was found to be ideal.

The efficacy of acephate + cartap mixture at reduced doses compared to the respective individual products in our trials *(Table 3)* suggests a synergistic action. However, further studies are required to establish the safety of this mixture to the parasitoid *C. plutellae*. The differential performance of DBM to commercial formulations of Btk in the laboratory and field studies suggest that injudicious use and sub lethal doses of Btk might unwittingly lead to the resistance of DBM to Btk toxins. Selection for resistance to one isolate of Btk might cause cross-resistance to other effective isolate of Btk. Such possibilities have been documented in *Plodia interpunctella* (Tabashnik, 1994).

At the farmer's level, presently, Btk is also being used as tank mix with the other commonly available insecticides. It is not desirable since Btk as a stand alone product is highly effective on DBM and tank mixture will not only add to the cost but also to excess loading of pesticide in the environment. Secondly, the aspects of compatibility and antagonism need a serious attention and detailed investigations are required before a combination using Btk is proposed.

Conclusion

Although Btk was found to be highly effective in the present field trials for the management of DBM, the reported resistance of DBM to Bt in other countries (Shelton *et al.*, 1993; Syed, 1992 and Tabashnik *et al.*, 1990) warrant a cautious approach to the resistance problem if Btk is to be used as the most successful insecticide for the management of DBM in India.

Since its approval in 1993 the present market size of Bt is about 40 tons of formulation. It is expected to touch 200 tons by the turn of this century. At this juncture, it is therefore essential that the enforcement authorities, manufacturers, extension workers and above all the farmers follow the strategies suggested below for effective management of DBM and also to avert development of resistance to the bio-pesticide.

a) Btk should be used judiciously not exceeding 2 sprays in a crop season, limiting its application to the critical stages of crop growth. Other promising conventional products like acephate, cartap, dichlorvos, carbosulfan etc., should be suitably alternated with Btk. No attempt should be made to introduce *B. thuringiensis* subsp. *aizawai* (Bta) for the next 10 years. Bta, if at all required should be brought into the country as an emergency when DBM develops high resistance to Btk.

b) The synthetic pyrethroids should not be recommended in areas where resistance is suspected.
c) Conservation of natural enemies in the field should be given utmost importance. Avoid insecticides that are highly toxic to the natural enemies.
d) Use of Indian mustard as trap crop should be promoted at all levels.

Acknowledgement

We thank the management of Rallis India Limited for the facilities provided in conducting the studies. Thanks are also due to M.S. Sastry for his valuable comments and editorial assistance.

References

Chand, P. and Choudhary, R. (1977). Patterns of insect plant relationships determining susceptibility of food plants in the diamondback moth *Plutella xylostella* (L) Curtis. *Mysore Journal of Agricultural Sciences* ***11****:* 547–49.

Dube, R.B. and Chand, P. (1977). Effect of food plants on the development of *Plutella xylostella* (L) (Lepidoptera; Plutellidae). *Entomon.* ***2*** *:* 139–40.

Feng, H.T. and Wang, T.C. (1984). Selectivity of insecticides to *Plutella xylostella* (L) and *Apanteles plutellae* Kurdj. *Plant Protection Bulletin of Taiwan. 26:* 275–84.

Jayarathnam, K. (1977). Studies on the population dynamics of the diamondback moth, *Plutella xylostella* (L) (Lepidoptera : Yponomeutidae) and crop loss due to the pest in cabbage. Ph.D. thesis, University of Agricultural Sciences, Bangalore 215 pp.

Lim, G.S., Sivapragasam, A. and Ruwaida, M. (1986). Impact assessment of *Apanteles plutellae* on diamondback moth using an insecticide - check method. Proceedings of the First International Workshop on Diamondback Moth Management, Taiwan, 1985. pp: 195 –204.

Shelton, A.M. Roberstson, J.L. Tang J.D., Perez, C. and Eigenbrode, S.D. (1993). Resistance of diamondback moth to *Bacillus thuringiensis* subspecies in the field. *Journal of Economic Entomology* ***86****:* 697–705.

Singh, S.P. and Singh, D. (1982). Influence of cruciferous host plants on the survival and development of *Plutella xylostella* L. *Journal of Research of Punjab Agricultural University.* ***19****:* 100–104.

Srinivasan, K. and Krishna Moorthy, P.N. (1992). Development and adoption of integrated pest management for major pests of cabbage using Indian mustard as a trap crop. Proceedings of the Second International Workshop on Diamondback moth,, Shanhua, Taiwan 1992, pp. 511–21.

Syed, A.R. (1992). Insecticide resistance in diamondback moth in Malaysia. Proceedings of the Second International Workshop on Diamondback moth, Shanhua, Taiwan 1992, pp. 437–42.

Tabashnik, B.E. (1994). Evolution of resistance to *Bacillus thuringiensis. Annual Review of Entomology* ***39****:* 47–79.

Tabashnik, B.E. Cushing, N.L. Finson, N. and Johnson, M.W. (1990). Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera : Plutellidae). *Journal of Economic Entomology* ***83****:* 1671–76.

Yadav, D.N. Patel, R.C. and Manjunath, T.M. (1975). Seasonal activity of *Apanteles plutellae* (Kurdj) a larval parasite of *Plutella xylostella* (L) at Anand (Gujarat, India). *Indian Journal of Plant Protection* ***3****:* 111–15.

Effects of *Bacillus thuringiensis* on eggs of three lepidopterous pests of crucifer vegetable crops

Liu Shu-Sheng and Zhang Guang-Mei

Department of Plant Protection , Zhejiang Agricultural University, Hangzhou 310029, China

Abstract

The insecticidal effect of a liquid of *Bacillus thuringiensis* (Bt) applied to the egg stage was studied in *Plutella xylostella*, *Spodoptera litura*, and *Pieris rapae* under laboratory conditions. In all of the three insect pests tested, Bt applied to the egg stage had no effect on eclosion, but caused substantial mortality of the larvae coming out of the Bt-treated eggs. With the recommended rates of concentration for field application, the rates of newly-emerged larvae reached 42%, 91% and 54% in the three insects, respectively. In *Plutella xylostella*, it was further determined that rates of mortality of larvae increased with increase of Bt dosage, but decreased with the age of eggs at treatment. The results of this study suggest that very high effect of Bt against these insect pests could be obtained in the field with applications timed at peaks of oviposition and eclosion.

Key words: *Bacillus thuringiensis*, effect, *Plutella xylostella*, *Spodoptera litura*, *Pieris rapae*, egg

Introduction

The microbial insecticide, *Bacillus thuringiensis* Berliner (Bt), has been used in the control of lepidopterous pests of crucifer vegetable crops for more than three decades. In recent years, the use of Bt in the control of lepidopterous pests of crucifer vegetable crops has been increasing rapidly owing to a combination of factors including insect resistance to synthetic chemical insecticides, public pressure to employ safer pest control measures, and improvement in the efficacy of Bt products. In fact, judicious use of Bt has proved to be an essential component of any successful program of integrated pest management in crucifer vegetable crops (Biever *et al.*, 1994; Yu *et al*., 1995; Andrews *et al*., 1992; Loke *et al*., 1992).

In the literature, evaluation of insecticidal activity of Bt has been largely directed to the larval stage (Chilcott and Wigley, 1994). However, it has been suspected that Bt applied to the egg and adult stages may also have insecticidal effects. Ali and Watson (1982) presented experimental evidence of effects of Bt on the egg and adult stages in the tobacco budworm, *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae). In all previous research of Bt against lepidopterous pests of crucifer crops, little effort has been directed to reveal insecticidal effects other than the direct kill of larvae (Liu *et al*., 1985). Lu *et al*. (1985) reported briefly that in the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) and the small cabbage white butterfly, *Pieris rapae* (Linnaeus) (Lepidoptera: Pieridae), Bt applied to the egg stage could cause substantial mortality of larvae emerging from the treated eggs, but the phenomenon was not examined in any detail. In order to provide a more thorough understanding of efficacy of Bt, we conducted a laboratory evaluation of the insecticidal effects of Bt applied to the egg stages of the diamondback moth, the cabbage butterfly and the cluster caterpillar, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). In the experiments with the diamondback moth, we further examined the effects in relation to dosage, egg age at treatment, and duration of immersion.

Materials and Methods

Bt formulation

The formulation used in this study was a Bt concentrate liquid of *B. thuringiensis* subsp. *kurstaki* containing 2500IU/mg, produced by the Hubei Bt Research and Development Centre, China. This liquid is a commercial Bt product which has been widely used on vegetable crops in China. In the experiments, the Bt liquid was diluted with distilled water to various concentrations as required.

All the experiments were conducted at 25±1 °C and 14 h photoperiod.

Experiments with diamondback moth

Culture of diamondback moth The stock culture of diamondback moth originated from a field sample of pupae collected from a cabbage plot in Hangzhou suburbs in September 1995. The culture was maintained on potted cabbage plants at 25±1 °C and 14 h photoperiod.

Method of egg collection The method described by Li *et al*. (1995) was followed to collect eggs of diamondback moth. A thin plastic bag was punched with tiny needles to get dense tiny holes (1–2 holes/ cm^2) on the bag. One cabbage leaf was inserted into the plastic bag. The opening of the bag was tied up with a rubber band to close up the cabbage leaf inside. The plastic bag containing the leaf was then hung inside an oviposition cage containing about 300 adults of diamondback moth. The adults laid their eggs on the surface of the plastic bag. The bag was taken out

of the cage 6 h after placement in the cage to obtain eggs of uniform age (0–6 h old, described as 0 h old below for simplicity). Eggs laid on the plastic bag could be clearly seen, and were cut into the form of egg cards containing about 25 eggs each ready for treatment.

Method of egg treatment and observation. Eggs were treated by immersion of the egg cards into Bt solutions for 10 seconds, except in the test of duration of immersion where different durations of 5–60 seconds were used (see *Table 3*). Upon removal from the Bt solution, the egg cards were dried by placing them on absorbent paper for about 2 minutes. The egg cards were then placed singly in Petri dishes and incubated at 25±1 °C and 14 h photoperiod. When the larvae were about to hatch, a fresh, clean leaf disc of cabbage was provided into each dish as food for the larvae. Observations were made daily to record the number of eclosion and dead larvae. The observations were terminated 3 days after the hatch of larvae.

Test of Bt dosage. Different levels of dosage were achieved by varying the rates of dilution of the Bt liquid with distilled water. Four levels of concentration and a control (distilled water) were used in the tests. The tests of each level of dosage were replicated ten times (see *Table 1*).

Effect of egg age. Newly-laid eggs were placed at 25±1 °C to develop to various ages before treatment with Bt solution. Three age classes, i.e., 0 h old, 24 h old and 48 h old, were tested. These three age classes approximated to the beginning, 30% and 60% of embryonic development. Egg treatment was done by immersion of egg cards of the appropriate ages into 500x Bt solution. Tests of each of the age classes and the control were all replicated ten times.

Effect of duration of immersion. Four different durations of 5–60 seconds were tested (see *Table 3*). Egg treatment was done by immersion of egg cards into 2000x Bt solution for appropriate durations as designed. Tests of each of the four duration treatments were replicated 12 times.

Experiments with cabbage butterfly and cluster caterpillar

Methods of insect rearing and egg collection One hundred plants of common cabbage planted in a greenhouse were inoculated with 50 pupae of the cabbage butterfly and 50 pupae of the cluster caterpillar. The adults of the two insects emerged and oviposited on the plants. At the peak of oviposition, eggs of the two insects were collected from the plants and taken to the laboratory.

Methods of egg treatment and observation Methods of egg treatment and observations were the same as those used for the diamondback moth. One level of dosage was tested in the cabbage butterfly *(Table 4)*. Three levels of dosage were tested in the cluster caterpillar *(Table 5)*.

Results

Effect of Bt applied to eggs of diamondback moth

Effect of different levels of Bt dosage. Mean rates of hatch at different levels of Bt dosage and the control were all very high (95–97%) and were nearly identical, indicating that Bt had no effect on eclosion when applied directly to the egg. However, rates of mortality of larvae emerging from the treated eggs increased significantly at all levels of dosage, by as much as about 40% at the two higher levels of dosage *(Table 1)*. *Table 1* also shows that rates of mortality were inversely correlated with rates of dilution of Bt from 1 000 x upwards, i.e., positively correlated with dosage. Further examination of the data revealed that 85% of deaths occurred on the first two days after eclosion, i.e., in the first instar.

Effect of egg age at treatment. Mean percentages of hatch in the three treatments and three controls ranged from 95% to 99%. Although the differences of percent hatch between treatment and control reached statistical significance in two of the three treatments, the differences were small and may be ignored biologically. However, rates of mortality of larvae reached 25–42% in the three treatments, while there were virtually no mortality of larvae in all the three controls *(Table 2)*. It can be seen that the rates of mortality decreased as the age at treatment increased.

Effect of duration of immersion. The results in *Table 3* show that within the range of 5–60 seconds, duration of immersion had no influence on the effects of Bt applied to the eggs.

Effect of Bt applied to the eggs of cabbage butterfly and cluster caterpillar

When the eggs of the cabbage butterfly received Bt treatment, percent hatch was similar to that of the control. However, 90.7% of the larvae emerging from the treated eggs died in the first to second instars, compared to 1.8% mortality in the control *(Table 4)*.

In the cluster caterpillar, treatment of the egg masses with three levels of dosage of Bt all resulted in about 50% of mortality of the larvae, again with no effect on the hatch of the eggs *(Table 5)*.

Discussion

The results of this study showed that, in all of the three insect pests tested, application of Bt to the egg stage does not affect eclosion, but can cause substantial mortality of the larvae out of the treated eggs. In this study, the deaths of larvae were caused by the consumption of a lethal dose during eclosion, because all larvae out of Bt-treated eggs were fed with fresh, clean (i.e., without Bt) cabbage leaves. It should be mentioned that 500 x solution of this Bt liquid is the recommended concentration used in field application with knapsack sprayers. This means that the high rates

Table 1. Effect of *Bacillus thuringiensis* at various dosages on the hatch of eggs and survival of newly-hatched larvae of diamondback moth

Dosage (Concentration)[a]	Percent hatch (Mean ± S.E.)[b]	Percent mortality of larvae (Mean ± S.E.)
500X	96.2 ± 1.4 a	41.9 ± 6.1 aAB
1 000X	96.8 ± 3.4 a	42.3 ± 3.4 aA
2 000X	96.2 ± 1.2 a	29.8 ± 3.3 bAB
4 000X	95.8 ± 1.6 a	12.9 ± 2.0 cC
CK	94.8 ± 1.1 a	2.3 ± 0.1 dD

[a] Dosage expressed in rates of dilution with distilled water. There were 10 replicates in each dosage and the control, 25 eggs in each replicate.
[b] Means in the same column followed by the same letter are not significantly different at 0.05 level (lower case) or 0.01 level (upper case), as determined by Duncan multiple range test.

Table 2. Effect of *Bacillus thuringiensis* on the hatch of eggs and survival of newly-hatched larvae of diamondback moth as affected by the egg age at treatment

Egg age at treatment (h)[a]	Percent hatch (Mean ± S.E.)[b]	Percent mortality of larvae (Mean ± S.E.)
0 h	96.1 ± 1.4 a	41.9 ± 6.1 aA
CK	94.8 ± 1.1 b	2.3 ± 0.1 cC
24 h	94.1 ± 1.0 b	31.1 ± 3.4 bAB
CK	96.7 ± 1.2 a	0.0 ± 0.0 cC
48 h	97.4 ± 1.0 a	24.7 ± 3.9 bB
CK	98.6 ± 0.7 a	0.5 ± 0.0 cC

[a]10 replicates in each age class and the corresponding control, 25 eggs in each replicate.
[b]Means in the same column followed by the same letter are not significantly different at 0.05 level (lower case) or 0.01 level (upper case), as determined by Duncan multiple range test.

Table 3. Effect of *Bacillus thuringiensis* on the hatch of eggs and survival of newly-hatched larvae of diamondback moth as affected by the duration of immersion of eggs

Duration of immersion in seconds[a]	Percent hatch (Mean ± S.E.)[b]	Percent mortality of larvae (Mean ± S.E.)
5	96.6 ± 1.3 a	27.6 ± 3.0 a
20	95.0 ± 1.1 a	25.8 ± 2.8 a
40	95.3 ± 1.9 a	25.1 ± 3.2 a
60	95.1 ± 1.2 a	25.4 ± 2.2 a

[a]12 replicates in each duration treatment, 25 eggs in each replicate.
[b]Means in the same column followed by the same letter are not significantly different at 0.05 level (lower case) or 0.01 level (upper case), as determined by Duncan multiple range test.

of mortality obtained in this study may occur in the field. With application of Bt onto plants, as is usual in field application, the newly-emerged larvae may continue to consume Bt on the leaves and thus may suffer further mortality. This would suggest that very high effect of Bt against these insect pests could be obtained with applications timed at the peak of oviposition and eclosion.

The results obtained with the diamondback moth suggest that the effect of Bt as an indirect ovicide may be influenced by Bt dosage and egg age at treatment. With the assumption in mind that the deaths are caused by consumption of Bt on the egg shell, the positive correlation between dosage and mortality seems to be straightforward. However, the negative correlation between ages of egg at treatment and mortality turned out to be the opposite of what was expected, because treatment at older ages was closer to eclosion and was expected to have stronger effect on the emerging larvae than treatment at younger ages. Possible explanations for the negative correlation could be (1) the shell of younger eggs was more sticky and retained more Bt toxin on the surface, and (2) the shell of younger eggs was more penetrable to Bt toxin and retained more Bt toxin inside the egg shell, or both of these two factors. Further studies are being conducted to reveal the factors.

Acknowledgement

This study forms part of an Australian-China cooperative project "Improvement of Integrated Pest Management of Brassica Vegetable Crops in China and Australia"(PN 9213), funded by the Australian Centre for International Agricultural Research.

References

Ali, A.S. A. and Watson, T. F. (1982). Effects of *Bacillus thuringiensis* var. *kurstaki* on tobacco budworm (Lepidoptera: Noctuidae) adult and egg stages. *Journal of Economic Entomology* ***75:*** 596–598.

Andrews, K. L., Sanchez, R. and Cave, R.D. (1992). Management of diamondback moth in Central America. In: *Diamondback Moth and Other Crucifer Pests:*

Table 4 Effect of *Bacillus thuringiensis* on the hatch of eggs and survival of newly-hatched larvae of *Pieris rapae*

Dosage (Concentration)[a]	Number of eggs treated	Percent hatch	Percent mortality of larvae
500X	140	85.0	90.7
CK	60	93.3	1.8

[a]Dosage expressed in rates of dilution with distilled water.

Table 5 Effect of *Bacillus thuringiensis* at various dosages on the hatch of eggs and survival of newly-hatched larvae of *Spodoptera litura*

Dosage (Concentration)[a]	Number of eggs treated	Percent hatch	Percent mortality of larvae
500X	224	100	53.6
1000X	332	100	47.9
2000X	208	100	53.4
CK	510	100	0.0

[a]Dosage expressed in rates of dilution with distilled water.

Proceedings of the Second International Workshop (ed. N. S. Talekar), pp. 487–497, Asian Vegetable Research and Development Centre, Tainan, Taiwan.

Biever, K.D., Hosttetter, S.L. and Kern, J.R. (1994). Evaluation and implementation of a biological control-IPM system for crucifers: 24-year case history. *American Entomologist*, ***40:*** 103–108.

Chilcott, C. N. and Wigley, P. J. (1994). Insecticidal activity of *Bacillus thuringiensis* crystal proteins. In*: Proceedings of the 2nd Canberra meeting on Bacillus thuringiensis* (ed. R. J. Akhurst), pp. 43–52, CSIRO Division of Entomology, Australia.

Li, Guang-Hong, Liang Dong-Rui, Meng Xiao-Lin, Zhu Ying, Cai Wang-Yang and Zhang Liang-Wu. (1995). A new method for collecting eggs of *Plutella xylostella* (L.). *Entomological Knowledge*, ***32:*** 172–173 (in Chinese).

Liu, Zhi-Cheng, Lu Ying—Xin, Miao Wen-Chao, Gao Jin-Siu and Chen Qi-Liang. (1985). Studies on the control of *Plutella xylostella* L. with *Bacillus thuringiensis* var *kurstaki*. *Acta Ptytophylacica Sinica*, ***12:*** 107–112 (in Chinese, English summary).

Loke, W.H., Lim, G.S., Syed,A.R. Abdul Aziz,A.M., Rani, M.Y., Jusoh, M. Md., Cheah, U.B. and Fauziah,I. (1992). Management of diamondback moth in Malaysia: development, implementation and impact. In: *Diamondback Moth and Other Crucifer Pests: Proceedings of the Second International Workshop* (ed. N. S. Talekar), pp. 529–539, Asian Vegetable Research and Development Centre, Tainan, Taiwan.

Lu Ying-Xin, Liu Zhi-Chen, Deng Yue-Jin, He Heng-Ping and Chen Qi-Liang. (1985). Effects of *Bacillus thuringiensis* on the old and infant larvae of *Pieris rapae* L. and *Plutella xylostella*. Nongye Weishenwu Tongxun *(Bulletin of Agricultural Microorganisms)*, ***5:*** 55–57 (in Chinese).

Yu, Zi-Niu, Luo Xi-Xia and Li Jian-Hong. (1995). Application of *Bacillus thuringiensis* preparations against crucifer insect pests in China. In: *ACIAR Workshop Report – Integrated Pest management in Brassica Vegetable Crops* (ed. Zhejiang Agricultural University), Hangzhou, 1995, pp. 31–35 (in Chinese, English summary).

Occurrence of a granulosis virus from two populations of *Plutella xylostella* (L.) in India

R. J. Rabindra, N. Geetha, S. Renuka, S. Varadharajan and A. Regupathy
Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore 641 003, India.

Abstract

Populations of the diamondback moth *Plutella xylostella* (L.) larvae from two locations : Ooty and Oddanchatram (Tamil Nadu State) were found to be infected by a granulosis virus (GV). The virus was highly pathogenic to *P. xylostella* larvae and was responsible for the collapse of laboratory colonies of the insect. Restriction endonuclease analysis of the viral DNA with Pst 1, Hind 3, Bam H1 and EcoR 1 showed similar profiles indicating that both the isolates of the GV are identical. Pst 1 produced the maximum number of 16 bands, Hind 3 showed 13 and Bam H1 and EcoR 1, both recorded 10 bands. The molecular weight of the viral DNA was found to be $64.05 \pm 2.07 \times 10^6$ daltons. Results of bioassays showed that the GV was highly pathogenic to *P. xylostella* larvae with an LC_{50} of 5.89 occlusion bodies/mm^2 of leaf surface for the second instar larvae. The LT_{50} was 5.67 days for the highest dose of 2.8×10^4 OB/mm^2 and it increased with decreasing concentration of the virus.

Key words: *Plutella xylostella*, granulosis virus, Restriction endonuclease analysis, bioassay.

Introduction

The diamondback moth, *Plutella xylostella* L. (Lepidoptera : Plutellidae) causes economic damage to cabbage, cauliflower and mustard in India and the cultivation of cauliflower as a seed crop is hampered by the heavy incidence of *P. xylostella* in Nilgris and Kodaikanal (Regupathy and Paranjothi, 1980). A granulosis virus (GV) infecting *P. xylostella* was first reported by Asayama and Osaki (1970) and the virus was subsequently found to be promising in the microbial control of the pest (Kao and Rose, 1976; Nakahara *et al*., 1986). After searching for several years we have isolated a GV from *P. xylostella* larvae for the first time in India and this paper deals with dosage and time mortality responses of *P. xylostella* larvae to the virus and the restriction endonuclease analysis of two isolates of the GV.

Material and Methods

Two laboratory colonies of *P. xylostella* maintained in the Department of Agricultural Entomology originally collected from Ooty and Oddanchatram were found to be heavily infected by granulosis virus. Diseased larvae were found to grow bigger than the healthy ones and were whitish in colour. Larvae in late stages of infection stopped feeding and upon death, dried down to scale-like structures closely adhering to the cauliflower leaves. Dark field microscopic examination of diseased larvae revealed thousands of capsules.

Both the colonies of *P. xylostella* were completely destroyed by the virus. The virus affected larvae from the two populations were collected separately and propagated by inoculating *P. xylostella* larvae by feeding cauliflower leaves treated with the respective GV suspension in distilled water containing 0.01 per cent Triton X 100. Diseased larvae were blended in distilled water and passed through several layers of cheese cloth and centrifuged at 2 500 rpm for three minutes to pellet unwanted insect debris. The supernatant was then centrifuged at 10 000 rpm for 30 min in a refrigerated centrifuge to pellet the virus. The pellets were washed twice with distilled water and finally suspended in distilled water. Counts of the occlusion bodies (OB) were made with the help of a Petroff Hauser and Helber counting chamber (depth 0.02 mm) under dark field microscopy. The virus was stored in a refrigerator at -18 °C until further use.

Restriction endonuclease analysis

Restriction endonuclease analysis of the two virus isolates were carried out following the method of Smith and Summers (1978) with some modifications. Aliquots of 120µl suspensions of the two viral isolates (ca 2.5×10^{10} OB) were taken in 1.5µl Eppendorf tubes and 25µl of 0.5m EDTA and 3µl of proteinase K were added and incubated at 37 °C for 90min. Then ca 75µl of 1 m Na_2 Co_3 was added and incubated at 37 °C for 15 min to release the virions from the OB. After adding 25µl of 10 per cent SDS, the samples were incubated at 37 °C for 30 min and centrifuged at 10 000 rpm for 3 min to remove any undissolved OB. The supernatant was extracted with an equal volume of tris-saturated phenol. The extraction was repeated using an equal volume of tris-saturated phenol : chloroform : isoamyl alcohol (25 : 24 : 1). Finally, the DNA was extracted with an equal volume of 24 : 1 chloroform : isoamyl alcohol. The extracted DNA was dialysed at 4 °C for 36 h changing the buffer three times. Samples of 25µl of DNA were digested with 1.5µl of Pst1, BamH1, Hind3 and EcoR1 along with appropriate buffers at 37 °C for 4 h and electrophoresed using 0.6 per cent agarose gel at 35 V and 50 MA current over night in a Biorad DNA sub cell system. Lamda DNA cut with Hind 3 and 1 kb ladder were used as markers. DNA bands stained with ethidium

bromide were photographed in a UV-transilluminator (UVP) using a Poloroid Camera (Copal, Ds34). The sizes of the different bands in the DNA were determined by comparing with the markers and the molecular weight determined by summation of the bands.

Bioassays

Since the restriction endonuclease analysis revealed that the virus obtained from both Ooty and Oddanchatram populations of *P. xylostella* were of the same strain *(Figure 1)*, bioassays to determine the LC_{50} and LT_{50} were carried out only with the virus isolated from Ooty population of *P. xylostella*. Cauliflower leaf discs were treated with 12µl suspension of GV (containing 0.01 per cent Triton x 100) of different concentrations to give 28 000, 2 800, 280, 28 and 2.8 OB/mm^2 of the leaf disc. The 12µl droplet of the virus suspension was placed on the centre of the leaf disc and spread uniformly over the entire surface of the leaf disc using a glass rod with a rounded and polished end. After the suspension had dried off, the discs were turned and the lower surfaces treated similarly. Control discs were treated with distilled water containing 0.01% Triton x 100 only. The leaf discs were placed in Petri dishes lined with wet filter paper discs and 35 second instar larvae of *P. xylostella* were released on the leaf discs in two replications. After 24 h of feeding, the larvae were removed to fresh untreated leaves of cauliflower. The leaves were changed daily and mortality data collected every day. The dosage and time mortality responses were subjected to probit analysis (Finney, 1962).

Results and Discussion

Restriction endonuclease analysis of the viral DNA of the two isolates of GV showed that there were no genetic variations in the profiles of PSt 1, Hind 3, Bam H1 and EcoR 1 *(Figure 1)*. Both the DNAs had identical profiles with no variations in the number or size of the bands. Pst 1 produced the maximum number of 16 bands, Hind 3 showed 13 and Bam H 1 and EcoR 1 both recorded 10 bands. The molecular weight of the viral DNA was found to be 64.05 ± 2.07 x 10^6 daltons. Results of the bioassay showed that the virus was highly pathogenic and the LC_{50} was found to be 5.89 occlusion bodies/mm^2 of the leaf surface *(Table 1)*. Probit analysis of time mortality responses revealed that the LT_{50} for a dose of 2.8 x 10^4 occlusion/mm^2 was 5.67 days and the values increased as the dose decreased *(Table 2)*. Abdul Kadir (1992) studying the potential of several baculoviruses for the control of *P. xylostella* reported the GV to be more virulent than either the NPV of *Galleria mellonella* or *Autographa californica*. The pest has developed resistance to several insecticides (Chawla and Kalra, 1976; Joia and Chawla, 1995) including the pyrethroids (Saxena *et al.*, 1989) in India. Chandrasekaran and Regupathy (1995 & 1996), Renuka and Regupathy, (1996) have monitored and documented the insecticide resistance in *P. xylostella* in Tamil Nadu. Since the GV is found to be highly pathogenic, there is scope for developing this virus as a biorational pesticide against the diamondback moth larvae.

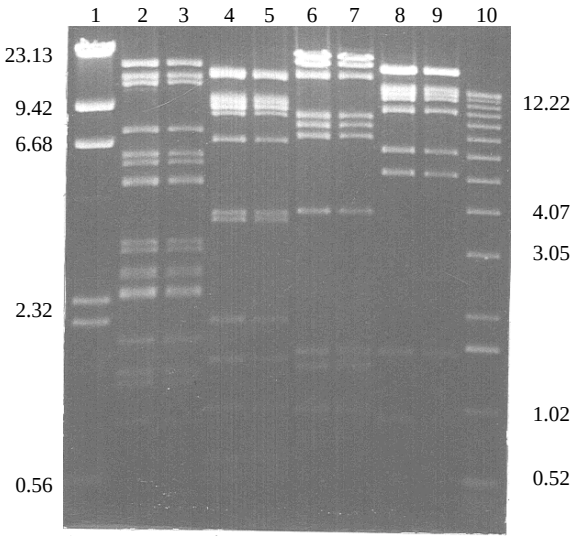


*Figure 1. Restriction endonuclease analysis of the DNA of Ooty (Tracks 2, 4, 6 and 8) and Oddanchatram (Tracks 3, 5, 7 and 9) isolates of GV of **P. xylostella** with Pst1 (Tracks 2, 3) Hind 3 (Tracks 4, 5) Bam H1 (Tracks 6, 7) and EcoR1 (Tracks 8, 9). Track 1 = λ DNA cut with Hind 3; 10 = Kb ladder*

Table 1. Probit analysis of dosage mortality response of second instar *P. xylostella* larvae to GV

Chi^2* (n-2)	Slope 'b'	LC_{50} OB/mm^2	Fiducial limits
1.096	0.2552	5.8919	0.9066 38.3728

*The line is significantly a good fit (P<0.05).

Table 2. Time mortality response of second instar larvae of *P. xylostella* to GV at different concentrations

Occlusion bodies/mm^2	Chi^2* (n-2)	b	LT_{50} (days)	Fiducial limits
28 000	3.25	5.1099	5.67	5.1966 6.1782
2 800	0.91	8.0231	7.0501	6.6492 7.4753
280	0.44	5.9312	7.8818	7.2183 8.6061
28	1.14	7.4198	9.2507	8.5635 9.9930
2.8	1.37	6.4034	11.0015	9.7990 12.3516

*All lines are significantly good fit (P<0.05).

Acknowledgement

The facility for the restriction endonuclease analysis of the viral DNA was set up in the Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, India under a collaborative project funded by the Natural Resource Institute, U.K. (Project No. 0047).

References

Abdul Kadir, H. (1992). Potential of several baculoviruses for the control of diamondback moth and *Crocidolomia binotalis* on cabbages. *In:* "*Diamondback moth and other crucifer pests*" (ed.N.S.Talekar). *Proceedings of the Second International Workshop*. Tainan, Taiwan, Asian Vegetable Research and Development Centre. 1990 pp. 185–192.

Asayama, T., and Osaki, N. (1970). A granulosis virus of the diamondback moth, *Plutella xylostella* (L.). *Journal of Invertebrate Pathology.* ***15:*** 284–286.

Chandrasekaran, J. and Regupathy, A. (1995). Status of insecticide resistance in field population of diamondback moth (DBM) *Plutella xylostella* in Tamil Nadu. *VI National Symposium on Integrated Pest Management. An entomological approach to sustainable agriculture*. Sanatan Dharm College, Muzaffarnagar. 1995, Abst.92.

Chandrasekaran, J. and Regupathy, A. (1996). Insecticide resistance in diamondback moth *Plutella xylostella* (L.) in India : Discriminating dose studies. *Proceedings of the XX International Congress of Entomology*. Florence, Italy 1996. Abstract No. 19–067.

Chawla, R.P. and Kalra, R.L. (1976). Studies on insecticide resistance in *Plutella xylostella* (Linn.) Diamondback moth. *Indian Journal of Plant Protection.*, ***4:*** 170–179.

Finney, D.J. (1962). A statistical treatment of the sigmoid response curve. *In:* "Probit analysis", (Edn. – 2), Cambridge University Press, London, 333 pp.

Joia, B.S. and Chawla, R.P. (1995). Insecticide resistance in diamondback moth, *Plutella xylostella* (L.) and its management in Punjab, India, *European Journal of Plant Pathology, XIII. International Plant Protection Congress*, The Hague, The Netherlands, 1995, Abstract No. 204.

Kao, H.W., and Rose, R.I. (1976). Effect of sunlight on the virulence of granulosis virus of the diamond back moth and evaluation of some protective adjuvants. *Plant Protection Bulletin* (Taiwan), ***18:*** 391–395.

Nakahara, L.M., McHugh, J.J.Jr., Otsuka, C.K., Fund Askai, F.Y., and Lai, P. (1986). Integrated control of diamondback moth and other insect pests using an overhead sprinkler system, an insecticide and biological control agents, on a watercress farm in Hawaii. In Diamondback moth management *Proceedings of first International Workshop*, AVRDC, Taiwan, pp. 403–413.

Regupathy, A. and Paranjothi.G.P. (1980). Evaluation of certain insecticides against diamondback moth *Plutella xylostella* on cauliflower seed crop. *Pesticides*, ***14:*** 30–31.

Renuka, S. and Regupathy, A. (1996). Monitoring of insecticide resistance in diamondback moth *Plutella xylostella* (L.) in Tamil Nadu. *Pesticide Research Journal* (In press).

Saxena, J.D., Rai, S.K., Srivastava, K.M. and Sinha, S.R. (1989). Resistance in the field populations of diamondback moth to some commonly used synthetic pyrethroids. *Indian Journal Entomology*, ***51:*** 265–268.

Smith, G.E. and Summers, M.D. (1978). Analysis of baculovirus genomes with restriction endonucleases. *Virology*, ***89:*** 517–527.

Pre-mortality effects of *Zoophthora radicans* infection in the diamondback moth

Michael J. Furlong, Giadi V. P. Reddy[1], Judith K. Pell and Guy M. Poppy

Entomology and Nematology Department, IACR-Rothamsted, Harpenden, Hertfordshire, AL5 2JQ, U. K.

[1]Present address: University of Agricultural Sciences-Dharwad, Agricultural Research Station, Hagari-583 138, Bellary District, Karnataka, India

Abstract

Larval food consumption and weight gain were not affected by *Z. radicans* until the third day after infection, one day prior to death from mycosis. No food was eaten on the day on which larvae died. Overall, infected larvae ate 45% less leaf tissue than uninfected larvae. Of the leaf tissue consumed by infected larvae, 87.5% was eaten on the first two days after infection and after this time infected larvae gained little weight. The efficiency with which ingested food was converted into body weight did not change as infection progressed. Infected female moths laid significantly fewer eggs than control moths. This difference was not only due to differential mortality between the infected and control treatments as egg production by infected females, prior to death (day 4 after infection), was significantly less than that of control females during the same period. Holding females for 24 h after eclosion (to allow further egg maturation), prior to infection, did not result in greater overall egg production when compared with moths infected on the day of eclosion. In olfactometer studies, *Z. radicans* infection in male moths did not affect the response to synthetic sex pheromone until the third day after inoculation when no individuals responded to pheromone. The response of *Z. radicans* infected male moths to mechanical stimulation was not affected until the third day after inoculation when infected individuals moved significantly shorter distances than controls. The number of infected female moths exposing their sex pheromone glands was significantly reduced by the third day after inoculation when compared with uninfected controls. Sex pheromone gland exposure by females and the number of male moths exhibiting wing fanning behaviour in response was positively correlated, indicating that gland exposure was an accurate method for assessing pheromone production.

Key words: *Plutella xylostella*, *Zoophthora radicans*, feeding, oviposition, sex pheromone.

Introduction

At IACR-Rothamsted, biological control strategies against *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) utilising the naturally occurring, environmentally safe, fungus *Zoophthora radicans* (Zygomycetes: Entomophthorales) have been developed (Furlong *et al.*, 1995; Furlong and Pell, 1996; Pell *et al.*, 1993a). The value of entomopathogens for insect pest control is often questioned as their slow mode of action frequently allows the pest species to almost complete its development on the crop before death occurs. *Zoophthora radicans* kills infected *P. xylostella* larvae and adults within 3–4 days of infection (Furlong *et al.*, 1995; Pell *et al.*, 1993b). This paper describes the effect of infection on *P. xylostella* larvae and adults as infection progresses, and the results are discussed in relation to IPM strategies.

Materials and Methods

Insect and fungal cultures

P. xylostella larvae from a laboratory culture were maintained on Chinese cabbage plants, *Brassica chinensis* var. *pekinensis* cv. Tip Top, at 23±2 °C. Two cultures were maintained, one under a photoperiod of 12: 12 h light: dark, and another 12 h out of synchrony under a photoperiod of 12: 12 h dark: light (the reverse photoperiod).

The *Z. radicans* isolate, NW250R, (Furlong *et al.*, 1995) was used in all experiments. The pathogen was stored and cultured on SEMA (SDA enriched with milk and egg yolk) as described by Furlong *et al.* (1995).

Preparation of mycelium mats

Mats of mycelium were prepared as described by Pell *et al.* (1993b); 3.5 cm diameter mats were produced for the feeding studies and 9 cm diameter mats for the oviposition and pheromone studies. All mats were placed in Petri dishes and held in humid conditions (100% RH) for 18 h prior to use to induce conidia production.

Effect of *Z. radicans* infection on *P. xylostella* larvae

Feeding and weight gain studies

Single squares of Chinese cabbage leaf (2 cm x 2 cm) were embedded in 1% tap water agar (TWA) in each of 28 Petri dishes (3.5 cm dia.). In 14 of the dishes the leaf square was inoculated with conidia produced by a mat of mycelium held in a Petri dish lid. The remaining 14 leaf squares were untreated. Single, pre-weighed, early third instar *P. xylostella* larvae were

then introduced onto the leaf surface in the inoculated and uninoculated Petri dishes. All 28 Petri dishes were incubated in humid conditions (100% RH) at 20 °C for 24 h. The larvae were then removed from the dishes, weighed individually and placed in labelled Petri dishes (3.5 cm dia.) containing single uninoculated fresh leaf squares (2 cm x 2 cm). This was repeated every 24 h until the larvae formed pupae or died from infection. The area of leaf eaten each day by individual larvae was measured by placing a grid (30mm x 30mm) over the surface of each leaf square. The weight of a unit area of leaf was calculated by weighing 5 leaf squares after they had been set in 1% TWA and held at 20 °C for 24 h; this was then used to calculate the weight of leaf tissue eaten by each larva on each day. The daily food conversion efficiency (FCE) was calculated [FCE=(larval weight gain/ weight of food consumed) x 100; Mohamed *et al.*, 1985].

Effect of *Z. radicans* infection on *P. xylostella* adults

Standardised inoculation of adult moths

Five moths were placed into individual Petri dishes (9 cm. dia.) and showered with conidia produced by a sporulating mat of mycelia held in the lid of the dish. The dishes were then held in humid conditions (100% RH) at 20 °C for 3 h to ensure infection.

Oviposition studies

Ten female moths which had recently emerged from pupal cases (3–6 h previously) were inoculated with *Z. radicans* conidia. These moths, together with 10 uninoculated female moths, were then placed individually in clean Petri dishes containing moistened filter paper (Whatman No. 1), a piece of Chinese cabbage leaf (≈40 cm^2), a food source (50% honey solution) and two 1-day old male moths. All 20 Petri dishes so prepared were held at 20ºC for 24 h. The three moths in each Petri dish were then introduced into another Petri dish (9 cm dia.) containing fresh leaf and a moistened filter paper. The number of eggs laid onto the individual leaf portions by each female was then counted; counts were made every 24 h until all individual females had died. Each day, control moths were handled prior to infected moths in order to minimise the possibility of cross infection. An identical experiment was set up simultaneously using female moths which had been held individually in glass tubes (7.5 cm x 1.0 cm) for 24 h post emergence.

Male response to synthetic sex pheromone

Fifty newly emerged (3–6 h previously) male *P. xylostella* adults from the reverse photoperiod culture were inoculated with *Z. radicans*. A four arm olfactometer was set up and illuminated only by an infra red light. A source of synthetic sex pheromone (0.1µg (Z)-11-hexadecenal, 0.1µg (Z)-11-hexadecenyl acetate, 0.001µg (Z)-11-hexadecanol in 0.5µl hexane) was held in a clean glass bottle connected by plastic tubing to one of the arms, each of the remaining three arms were similarly connected to empty clean glass bottles. A vacuum pump was used to draw air through the apparatus. At the onset of scotophase on the day that the moths emerged five uninoculated moths were introduced to the central chamber of the olfactometer and air drawn through the system. The number of moths leaving the central chamber in an eight minute period was recorded. The moths were carefully removed, replaced by five inoculated moths and the procedure repeated. The experiment was replicated five times. On removal from the olfactometer, inoculated and uninoculated moths were returned to their respective holding boxes and incubated at 20 °C. The experiment was repeated, on the same individuals, every 24 h until inoculated moths died from mycosis.

Male response to mechanical stimulation

Ten newly emerged male *P. xylostella* adults were inoculated with *Z. radicans* conidia. Individual moths were then placed into 10 separate Petri dishes (9 cm dia.). Ten Petri dishes containing individual uninoculated moths were also set up and all the dishes were held at 20 °C for 24 h. The lid of one Petri dish was lifted slightly, the moth prodded with a blunt forceps and its movement traced onto the lid of the dish with a fine marker pen. The movement in response to prodding of all twenty moths was recorded. The experiment was repeated every 24 h until inoculated moths died from mycosis. An image analyser (Magiscan, M2A) was used to measure the length of all the traces.

Ability of females to "Call"

One hundred and twenty newly emerged female *P. xylostella* adults were inoculated with *Z. radicans* conidia. Thirty inoculated moths were then introduced to each of 4 clear plastic ventilated boxes containing honey solution (50%) as a food source. Four identical boxes each containing 30 uninoculated moths were also set up. The 8 boxes were arranged randomly on a bench in 20 °C constant environment room and the number of individuals exposing their pheromone glands 1 h before the beginning of scotophase and 1 h into photophase was recorded. Four muslin covered open ended glass tubes (10 cm x 2 m) each containing ten 1 day old male moths were placed on the ventilation holes of each of the boxes. The number of individuals exhibiting wing fanning behaviour in the ten minute period immediately following the female pheromone gland exposure assessment was recorded.

Results

Feeding and weight gain studies

All *P. xylostella* larvae infected with *Z. radicans* died from mycosis on the fourth day of infection while all control larvae successfully pupated. Prior to pupation or death, control and infected larvae consumed an average of 238.79 (±17.90) and 130.36 (±14.14) mm^2 of leaf tissue respectively *(Fig. 1a)*; this difference was highly significant ($F_{1,26}$= 22.661; p< 0.001) and represented a reduction of 45% in leaf consumption by infected larvae compared with control larvae.

Analysis of the data on a daily basis on the first 4 days after infection showed that fungal infection significantly reduced leaf consumption ($F_{1,106}$= 28.37; p< 0.001; *Figure 1b*). The quantity of leaf consumed also varied significantly over time in both treatments ($F_{1,106}$= 55.49; p< 0.001), with the greatest quantity 101.43 (±10.33) mm^2 and 90.00 (±11.69) mm^2 for control and infected larvae respectively) being consumed in each treatment on the second day of the experiment *(Figure 1b)*. The interaction term between treatment and time after infection was also significant ($F_{3,106}$= 6.54; p< 0.001), indicating that the rate of feeding declined more rapidly in infected larvae than in control larvae *(Figures 1a* and *1b)*. There was no significant difference between the quantities of leaf consumed by infected and control larvae until the third day after infection when control larvae consumed significantly more leaf than infected larvae (Tukey's test, p< 0.01). Infected larvae did not feed at all on the fourth day after infection, the day on which they died.

There was a significant difference in weight gain between infected and control larvae over the course of the infection period ($F_{1,78}$= 9.04; p< 0.01; *Figure 1c*). There was also a significant difference in the weight gain of larvae between successive days ($F_{2,78}$= 17.29; p<0.001), although there was no significant difference between the weight gain of control larvae on the second and third days after infection (Tukey's test, p>0.05). The interaction term between treatment and time after infection was significant ($F_{2,78}$= 5.69; p< 0.01), indicating that weight gain varied differentially between treatments over time. The weight gain by control and infected larvae was not significantly different until the third day after infection when control larvae gained significantly more weight than infected larvae (Tukey's test, p< 0.01). On each of the first three days after infection there was no difference between the Food conversion efficiency (FCE) for control and *Z. radicans*-infected larvae *(Figure 1d).*

Effect of *Z. radicans* infection on *P. xylostella* adults

Oviposition studies

All moths infected with *Z. radicans* died from mycosis on the fourth day of infection but all control moths were alive when the experiment was terminated after 12 days. Analysis of the total number of eggs laid per moth by day 12 showed that there was a significant difference between treatments ($F_{3,32}$= 10.35; p< 0.001). Comparison of means tests showed that the two age classes of control moths did not lay significantly different numbers of eggs by this time (Tukey's test, p> 0.05). However, both age classes of infected moths laid significantly fewer eggs than their relevant controls (Tukey's tests, p< 0.01; *Figures 2a* and *2b)*. This represents an overall reduction in number of eggs laid of 72% and 65% by the moths infected on the day of eclosion and one day post-eclosion, respectively.

The number of eggs produced per moth on the first four days after infection was analysed separately for each age class. Moths infected on the day of eclosion laid significantly fewer eggs than control moths of an identical age ($F_{1,64}$ = 5.48; p< 0.05; *Figure 2c*). There was no significant difference between the total number of eggs laid on each of the 4 days ($F_{3,64}$ = 1.41; p> 0.05) nor was there a significant interaction between treatment and time after infection ($F_{3,64}$ = 1.62; p>0.05). Moths infected one day post-eclosion laid significantly fewer eggs than control moths of an identical age ($F_{1,64}$ = 6.83; p< 0.05; *Figure 2d*). There was a significant difference between the number of eggs laid on different days ($F_{3,64}$ = 3.61; p< 0.05); significantly more eggs being laid on the first day (Tukey's test, p< 0.05). There was no significant interaction between treatment and time after infection ($F_{3, 64}$= 0.12, p> 0.05). In both age class treatments comparison of means tests showed that there was no significant difference between the numbers of eggs laid by control and infected individuals until the third and fourth days after infection when infected moths laid significantly fewer eggs than control moths (Tukey's tests, p< 0.05 and p< 0.01, respectively).

Male response to synthetic sex pheromone

More control moths moved out of the central chamber of the olfactometer in response to pheromone than infected moths ($F_{1,38}$ = 12.0, p< 0.01). Comparison of means tests showed that there was no difference in the response of infected and healthy moths on the first two days after infection (Tukey's test, p> 0.05). On the third day after infection no infected moths left the central chamber of the olfactometer.

Male response to mechanical stimulation

Overall, infected moths moved less than control moths after mechanical stimulation ($F_{1,72}$ = 18.9). Comparison of means tests showed that there was no difference between infected and control moths in the response to mechanical stimulation until the third day after infection when infected moths moved significantly less after mechanical stimulation than control moths (Tukey's test, p<0.05).

Ability of females to "Call"

Overall there was a significant difference between the number of healthy and infected female moths exposing their pheromone glands ($F_{1,36}$ = 116.1, p< 0.001). More moths exposed their pheromone glands in the evening than in the morning ($F_{1,36}$ = 80.2, p< 0.001). As time after infection increased there was a significant decrease in the number of female moths exposing their pheromone glands ($F_{2,36}$ = 123.9, p< 0.001). Comparison of means tests showed that this effect became significant on the third day after infection (Tukey's test, p< 0.05).

Significantly more male moths were induced to wing fan by healthy female moths than by infected moths ($F_{1,36}$ = 44.5, p< 0.001). More male moths fanned in response to female moths in the evening than in the morning ($F_{1,36}$ = 32.1, p< 0.001). As time after

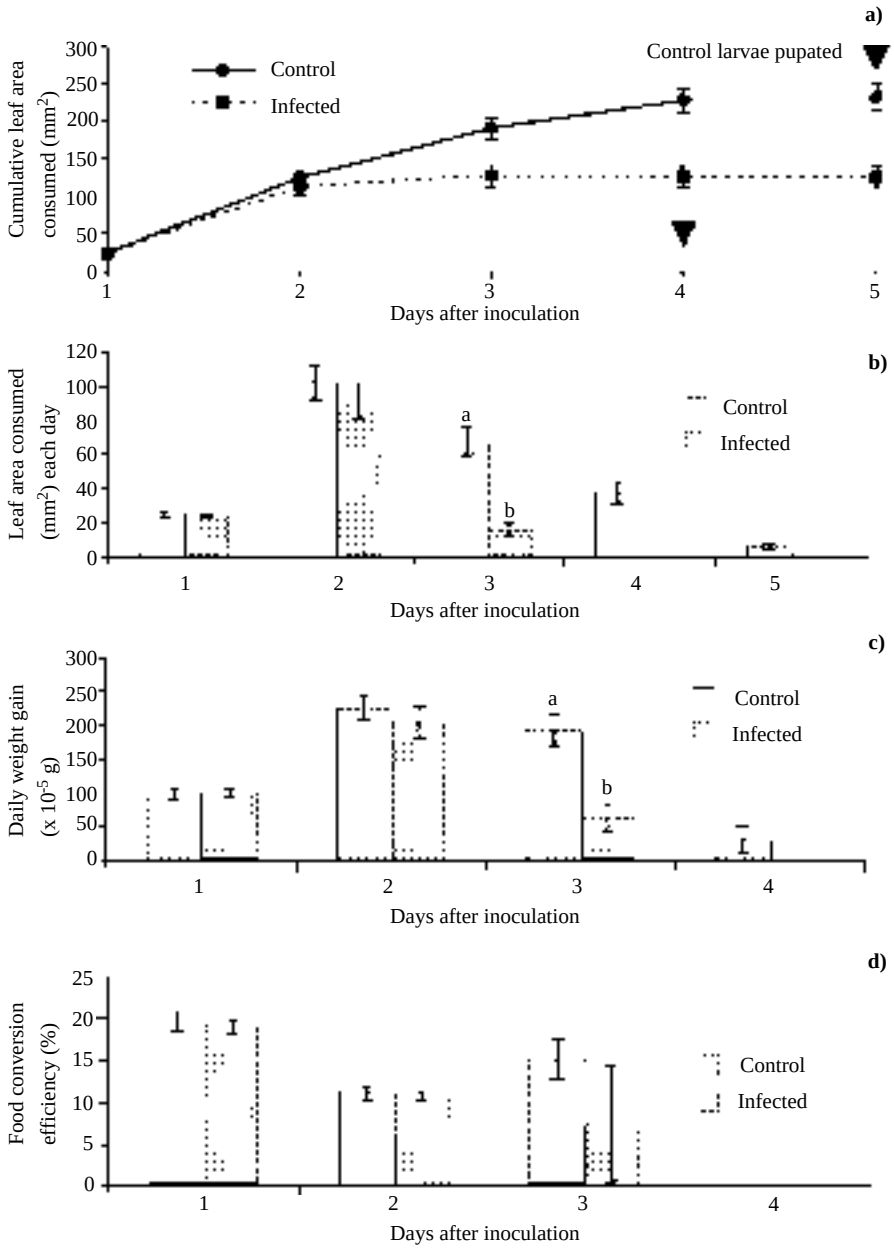


Figure 1 a). Cumulative area of cabbage leaf consumed by Zoophthora radicans-infected and uninfected Plutella xylostella larvae. b). Daily cabbage leaf consumption by Zoophthora radicans-infected and uninfected Plutella xylostella larvae. c). Weight gain by Zoophthora radicans-infected and uninfected Plutella xylostella larvae. d). Daily food conversion efficiency of Zoophthora radicans-infected and uninfected Plutella xylostella larvae.
Columns marked with different letters on the same day after infection are significantly different from each other (Tukey's test $p < 0.01$).

infection of the female moths increased there was a significant decrease in the number of male moths exhibiting wing fanning behaviour ($F_{1,36} = 48.3$, $p < 0.001$). Comparison of means tests showed that this effect became significant on the third day after infection (Tukey's test, $p < 0.05$). Linear regression of the number of females exposing their pheromone glands against the number of males wing fanning was highly significant ($F_{1,14} = 413.7$, $p < 0.001$; $r^2 = 0.967$).

Discussion

There are no visible effects of *Z. radicans* infection in *P. xylostella* larvae until a few hours before death when the body colour changes from emerald green to buff-yellow. Infected larvae consumed 45% less leaf tissue than healthy, control larvae and 87.5% of the tissue eaten by infected larvae was consumed within 2 days of infection *(Figure 1a)*. Infected larvae effectively stop feeding 2 days after infection. This counters the frequent criticisms that biological control of insects using fungal agents is slow and allows the pest to almost complete its development before death. Larvae were infected early in the third instar. Leaf area consumed and larval weight gain for healthy and infected individuals reached a peak on the second day after infection, which represents feeding and growth during the middle of the third instar *(Figures 1a, 1b* and *1c)*. Infection of larvae earlier in the life cycle would result in death occurring at an earlier stage

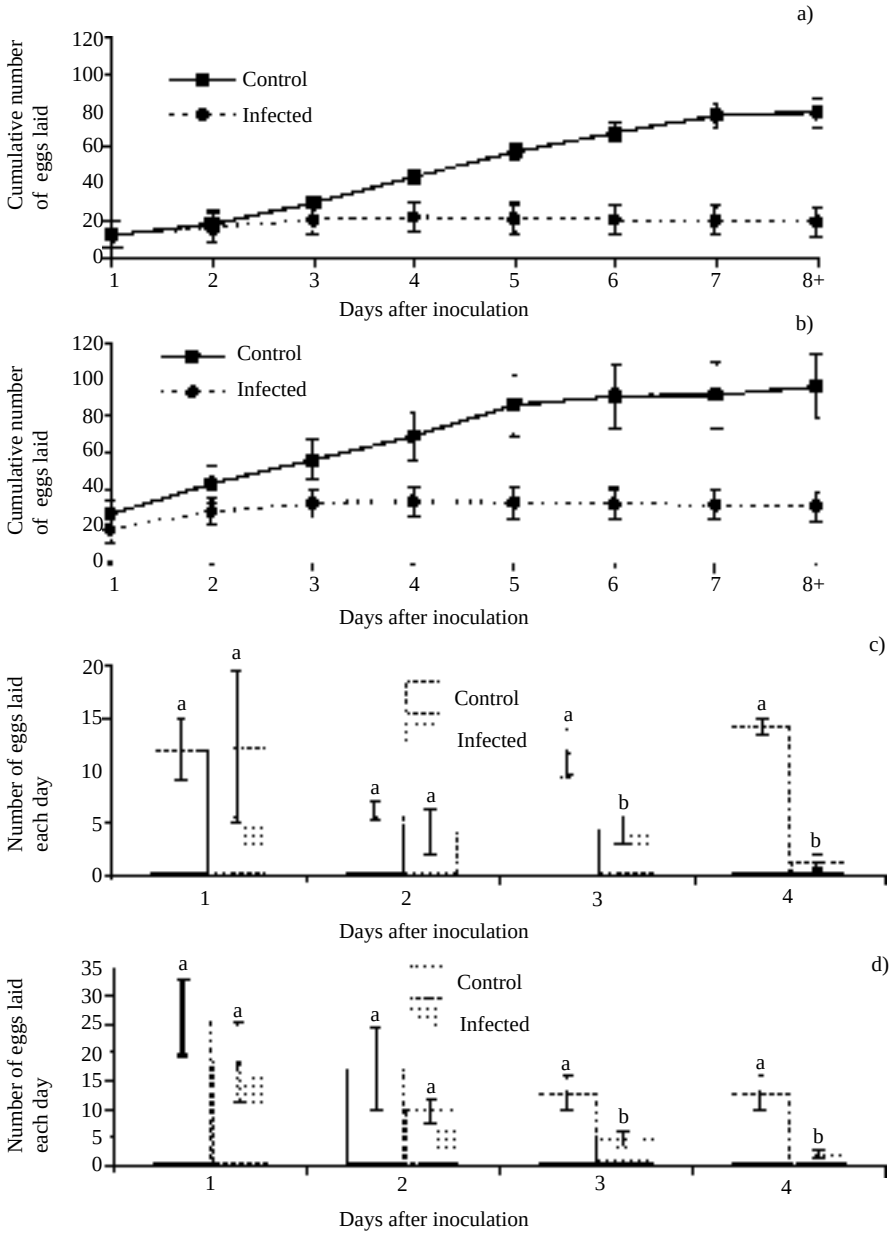


Figure 2 a). Cumulative oviposition by female Plutella xylostella infected with Zoophthora radicans on the day of eclosion and uninfected females of an identical age. b). Cumulative oviposition by female Plutella xylostella infected with Zoophthora radicans one day post eclosion and uninfected females of an identical age. c). Number of eggs laid by female Plutella xylostella infected with Zoophthora radicans on the day of eclosion and uninfected females of an identical age. d). Number of eggs laid by female Plutella xylostella infected with Zoophthora radicans one day post eclosion and uninfected females of an identical age.
Columns marked with different letters on the same day after infection are significantly different from each other (day 3 after infection Tukey's test, p< 0.05: day 4 after infection Tukey's test, p< 0.01).

leading to a further reduction in total food consumption.

Previous workers (Tyrell, 1990; Mohamed *et al.*, 1985; Hajek, 1989) have shown that entomophthoralean infections in lepidopteran larvae can decrease food consumption. Hajek (1989) suggested that this is due to protoplast proliferation in the haemolymph which disrupts metabolic processes and reduces the efficiency of food utilisation. However, in this study the food conversion efficiency of *Z. radicans*-infected and control *P. xylostella* larvae did not differ from each other as the infection progressed *(Figure 1d)*, although food consumption and weight gains decreased *(Figures 1a, 1b* and *1c)*. This indicates that there was no decrease in the efficiency of food utilisation by *Z. radicans*-infected *P. xylostella* and it is more likely that the decrease in feeding activity during the 24 h prior to death is due to a less subtle effect such as the invasion of vital organs by hyphal bodies.

Zoophthora radicans infection significantly reduced the number of eggs laid by female *P. xylostella*. A component of this difference is accounted for by the eggs laid by uninfected females after the infected moths died from disease *(Figures 2a* and *2b)* and, as such, can be explained in terms of

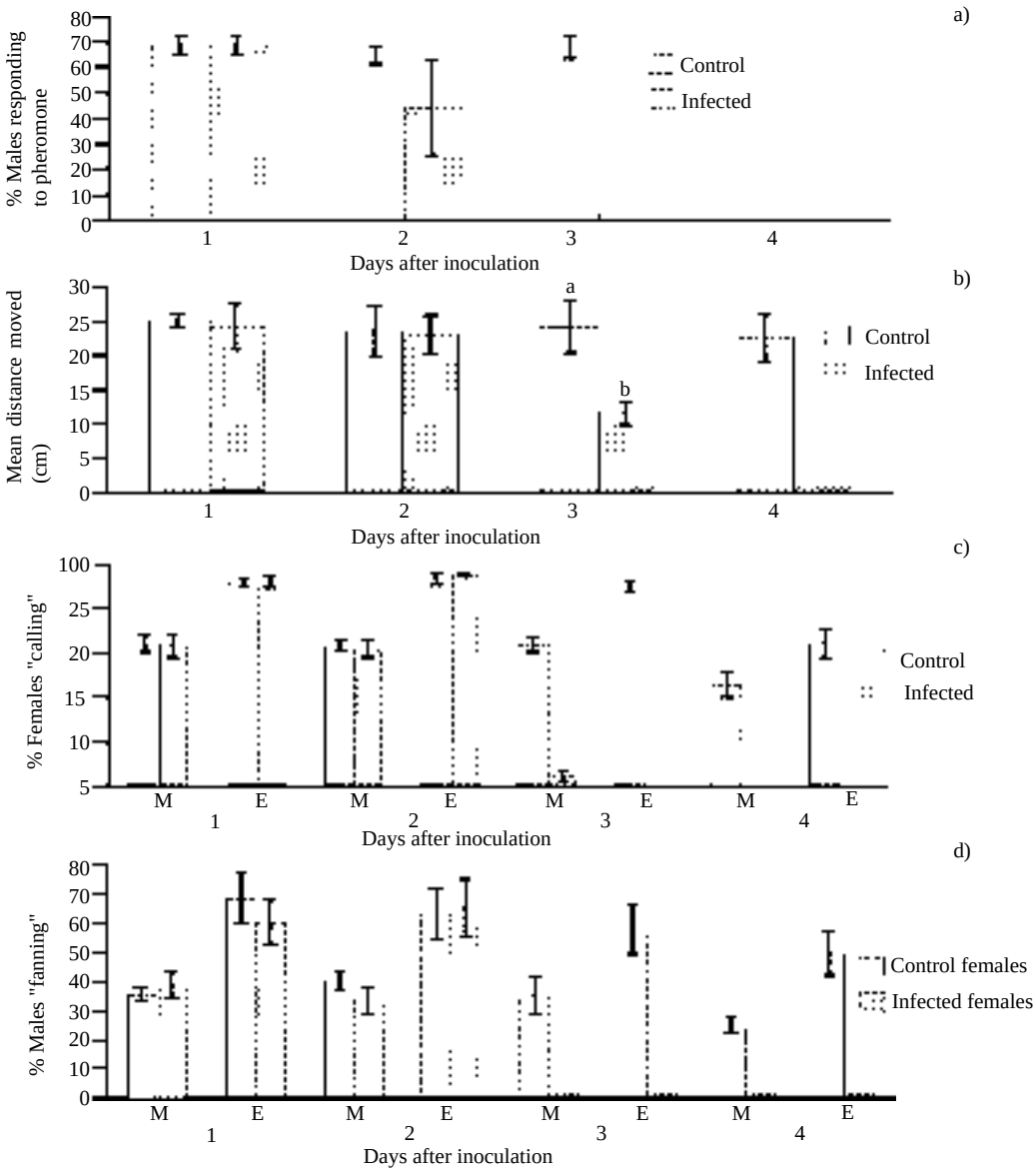


Figure. 3a). Percentage of male Plutella xylostella infected with Zoophthora radicans and uninfected males responding to synthetic sex pheromone in an olfactometer. b). Mean distance moved by male Plutella xylostella infected with Zoophthora radicans and uninfected males in response to mechanical stimulation. Columns marked with different letters on the same day after infection are significantly different from each other (Tukey's test p< 0.05). c). Percentage of female Plutella xylostella infected with Zoophthora radicans and uninfected females exposing their sex pheromone glands one hour into photophase (M) and one hour prior to the onset of scotophase (E). d). Percentage of male Plutella xylostella exhibiting fanning behaviour in response to Zoophthora radicans-infected and uninfected female Plutella xylostella.

the differential mortality between the infected and control treatments. However, the number of eggs laid by infected moths in both age classes prior to death was significantly reduced compared with controls. During the two days immediately after infection there was no decrease in daily egg production by infected females from either age class when compared to the relevant controls. However, on the third day post infection oviposition by infected females in both age classes was significantly reduced. This result is similar to the results in the feeding studies and is also probably due to the invasion of the vital organs by hyphal bodies.

Holding moths for one day before infection did not enable these individuals to lay a greater number of eggs than individuals infected immediately upon eclosion. Although individuals which were held for one day post eclosion laid more eggs on the first day during which they were able to oviposit than on subsequent days, their total egg production was no greater than that of individuals which were allowed to oviposit immediately upon eclosion. This suggests that *Z. radicans* infection had no effect on the ability of adults to develop and lay eggs in the first few days after infection. Only when infection was relatively well advanced (day 3 post infection) were oviposition rates reduced.

The response of infected male moths to synthetic sex pheromone and mechanical stimulation was not affected on the first two days after infection. On the third day no individuals responded to the pheromone

stimulus but the response to mechanical stimulation was reduced by only 50%. Thus, although individuals were capable of moving they did not in response to pheromone. It is possible that in the latter stages of infection the ability of the moth to perceive pheromone is inhibited, however, lethargy induced by the partial invasion of vital organs by hyphal bodies is a more likely explanation for the lack of response. In common with the other studies reported, exposure of the sex pheromone glands by infected female moths was not affected until the third day after infection. The highly significant regression of the number of females exposing their sex pheromone glands against the number of males exhibiting wing fanning behaviour indicated that gland exposure signified pheromone production. Although, in the latter stages of infection the chemical pathways for the synthesis of pheromone may be inhibited a more likely explanation is that mechanical disruption of tissues reduces the moth's vitality.

The results clearly show that the pre-mortality effects of *Z. radicans* infection in *P. xylostella*, although not immediately apparent, can be considerable. Overall feeding and oviposition rates can be drastically reduced and the response to and production of sex pheromone by infected male and female moths, respectively, can be inhibited. The ability to induce such reductions in larval voracity and adult fecundity and physiology must have an important role to play in pest management systems into which *Z. radicans* can be integrated and merits further investigation.

Acknowledgement

MJF was funded by the Leverhulme Trust and GVPR by Rothamsted International. IACR-Rothamsted receives grant-aided support from the Biotechnology and Biological Sciences Research Council of Great Britain.

References

Furlong, M. J. and Pell, J. K. (1996). Interactions between the Fungal Entomopathogen *Zoophthora radicans* Brefeld (Entomophthorales) and Two Hymenopteran Parasitoids Attacking the Diamondback Moth, *Plutella xylostella* L. *Journal of Invertebrate Pathology.* ***68:*** 16–21.

Furlong, M. J., Pell, J. K., Ong, P. C. and Syed, A. R. (1995). Field and laboratory evaluation of a sex pheromone trap for the autodissemination of the fungal entomopathogen *Zoophthora radicans* by the diamondback moth, *Plutella xylostella. Bulletin of Entomological Research.* ***85(3):*** 331–337.

Hajek, A. E. (1989). Food Consumption by *Lymantria dispar* (Lepidoptera: Lymantriidae) Larvae Infected with *Entomophaga maimaiga* (Zygomycetes: Entomophthorales). *Environmental Entomology.* ***18(4):*** 723-727.

Mohamed, A. K. A., Lewis, L. and Lewis, D. (1985). The effect of *Erynia radicans* on food consumption, utilisation and fecundity by the Spruce Budworm *Choristoneura fumiferana*. *In:* "Proceedings Symposium: Microbial control of spruce budworms and gypsy moths" General Technical Report NE-100, pp. 77–81 Forest Service, USDA.

Pell, J. K.,Macauly, E. D. M. and Wilding, N. (1993a). A pheromone trap for the dispersal of the pathogen *Zoophthora radicans* Brefeld. (Zygomycetes: Entomophthorales) amongst populations of the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Yponomeutidae). *Biocontrol Science and Technology.* ***3:*** 315-320.

Pell, J. K., Wilding, N., Player, A. L. And Clark S. J. (1993b). Selection of an Isolate of *Zoophthora radicans* (Zygomycetes: Entomophthorales) for Biocontrol of the Diamondback Moth *Plutella xylostella* L (Lepidoptera: Yponomeutidae). *Journal of Invertebrate Pathology*. ***61:*** 75–80.

Tyrell, D. (1990). Pathogenesis of *Entomophaga aulicae* I. Disease Symptoms and Effect of Infection on Weight Gain of Infected *Choristoneura fumiferana* and *Malacosoma disstria* Larvae. *Journal of Invertebrate Pathology.* ***56:*** 150–156.

Integration of *Zoophthora radicans* and synthetic female sex pheromone for the control of diamondback moth

Michael J. Furlong and Judith K. Pell

Entomology and Nematology Department, IACR-Rothamsted, Harpenden, Hertfordshire, AL5 2JQ, U. K.

Abstract

The widespread fungal pathogen *Zoophthora radicans* kills both adult and larval stages of the diamondback moth. At IACR-Rothamsted, a novel strategy for diamondback moth control, based upon a fast entry/slow exit trap containing the female sex pheromone and the pathogen, is under development. Male moths are attracted into an inoculation chamber where they are showered with infective conidia of *Z. radicans*. On habituation to the pheromone, they leave the trap and re-enter the crop, passively vectoring conidia to other susceptible adults and larvae and creating further foci of infection when they succumb to disease.

In field (Cameron Highlands, Malaysia) and laboratory trials, moths were attracted to the trap at all times of the day, spent long enough in the trap to acquire a lethal infection and were killed by *Z. radicans* infection within their demonstrated longevity in the field. The influence of rain, sunlight and temperature on conidial persistence was also evaluated.

At high doses *Z. radicans* is pathogenic to the parasitoid *Diadegma semiclausum* but not to *Cotesia plutellae*. This physiological susceptibility is unlikely to translate into susceptibility in the field. Neither parasitoid vectored *Z. radicans* conidia to susceptible larvae but host movement evoked by *D. semiclausum* foraging rendered larvae more likely to encounter infective conidia.

Key Words: Pheromone, *Zoophthora radicans*, persistence, *Diadegma semiclausum*, *Cotesia plutellae*.

Introduction

Zoophthora radicans Brefeld (Entomophthorales) is a virulent fungal entomopathogen which has been reported to cause epizootics in populations of the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae) throughout the world (Aruta *et al*., 1974; Kanervo 1946; Ooi, 1981; Riethmacher *et al*., 1992; Ullyet and Schonken, 1940) The pathogen regularly causes epizootics in *P. xylostella* populations in the Cameron Highlands of Malaysia and several strains have been isolated and their virulence quantified (Pell *et al*., 1993b). A highly pathogenic strain, NW250, is being developed as part of a novel strategy for *P. xylostella* control at IACR-Rothamsted (Furlong *et al*., 1995). Male moths are attracted into a specially designed pheromone trap (Pell *et al.*, 1993a) where they are inoculated with *Z. radicans* primary conidia produced by mats of mycelium. On habituation to the pheromone the moths leave the trap and re-enter the crop. When an individual dies from *Z. radicans* infection primary conidia are actively discharged from the cadaver. In conditions of high humidity, these conidia germinate to produce secondary conidia (capilliconidia). For the strategy to work, inoculum mechanically vectored from the trap or produced from the cadavers of infected male moths must remain in a viable/infective state on the cabbage crop or on the surface of the soil until it is encountered by other susceptible *P. xylostella* individuals. The retention of the ability to infect *P. xylostella* by conidia on the surface of foliage and soil in the Cameron Highlands has been investigated and, in laboratory studies, the relative importance of radiation, fluctuating temperatures and rainfall determined.

The importance of the endolarval parasitoids *Diadegma semiclausum* (Hymenoptera: Ichneumonidae) and *Cotesia plutellae* (Hymenoptera: Braconidae) in regulating *P. xylostella* populations is well documented (Talekar and Griggs, 1986; Talekar, 1992). As the parasitoids forage for host larvae on the cabbage crop they will come in close proximity to *Z. radicans* introduced for *P. xylostella* control. The susceptibility of adults of both species of parasitoid to *Z. radicans* infection relative to adult and larval stages of *P. xylostella* has been determined and the potential of both species to vector infective conidia to susceptible hosts investigated.

Materials and Methods

All field experiments were performed at MARDI's Cameron Highland field station. The pheromone trapping experiments relied on natural field populations of *P. xylostella* and laboratory studies assessing the field persistence of *Z. radicans* conidia utilised insects from the "SS" *P. xylostella* strain maintained at MARDI. All other laboratory studies were performed in the U. K. and utilised *P. xylostella* from a strain which has been in culture at IACR-Rothamsted for over 5 years. *D. semiclausum* and *C. plutellae* were field collected in the Cameron Highlands. The *Z. radicans* strain NW250R was used in all experiments, this was originally isolated from *P. xylostella* in the Cameron Highlands (Pell *et al.* 1993b) and was cultured as described by Furlong *et al.* (1995).

Pheromone trap evaluation

The periodicity of response to synthetic sex pheromone by male moths, the time spent inside the inoculation chamber of the prototype pheromone trap by male moths, the field longevity of male moths and the time taken for *Z. radicans* infection to kill male moths was investigated in the field (Furlong *et al.*, 1995). In the laboratory, bioassays determined the length of time male moths must spend inside the inoculation chamber of the pheromone trap in order to acquire a lethal infection of *Z. radicans* (Furlong *et al.*, 1995).

Persistence of Z. radicans conidia

The effect of field exposure on the infectivity of *Z. radicans* conidia on soil and foliage was investigated (Furlong and Pell, 1996a). In the U.K., supplementary laboratory studies examined the effect of temperature fluctuation, rainfall, exposure to ultra violet radiation, exposure to simulated tropical sunlight and soil water content on the infectivity of conidia (Furlong and Pell, 1996a).

Interactions between the pathogen and important parasitoids of the diamondback moth

The susceptibility of adult *C. plutellae* and *D. semiclausum* to *Z. radicans* infection was quantified relative to the susceptibility of adult and larval stages of *P. xylostella.* The ability of each species of parasitoid to vector conidia to healthy *P. xylostella* larvae was investigated and video equipment was subsequently used to quantify larval behaviours in the presence and absence of both species of parasitoid. Larval movement in the presence and absence of parasitoids was then correlated with *Z. radicans* infection rates in *P. xylostella* larvae (Furlong and Pell, 1996b).

Results

Pheromone trap evaluation

Synthetic pheromone lures attracted adult male *P. xylostella* at all times of the day *(Table 1)*. Moths spent a mean time of 88 seconds within the inoculation chamber of the trap *(Table 1)*. The field longevity of male moths was 4.9 (±0.3) days and it took 3–4 days before infected individuals died in the field *(Table 1)*. Laboratory bioassays showed that if inoculum was held above the pheromone lure in the trap the LT_{50} for a single visit to the inoculation chamber by male moths was 303 (126–630) seconds. When the amount of inoculum was doubled (inoculum held above and below the pheromone lure) the LT_{50} was reduced to <60 seconds *(Table 1)*.

Persistence of Z. radicans conidia

The ability of *Z. radicans* conidia to infect *P. xylostella* was dramatically reduced after just 24 h field exposure in the Cameron Highlands, Malaysia. In laboratory studies some conidia retained the ability to infect for up to 16 days on the surface of foliage or soil *(Figures 1a* and *1b)*. The rate at which conidia lost the ability to infect was greater on the adaxial leaf surface than on the abaxial leaf surface *(Figure 1a)* and greater on soil with a low moisture content than on soil with a higher moisture content *(Figure 1b)*. One hour of simulated heavy rainfall had no impact on the number of infective primary conidia remaining on either the adaxial or abaxial leaf surface, but 3 h rainfall significantly reduced the number of infective conidia remaining on the adaxial leaf surface *(Figure 1c)*. When primary conidia were incubated on leaf surfaces in conditions of high humidity, to encourage capilliconidia development, and then subjected to heavy rainfall, 1 h of exposure significantly reduced the number of infective conidia remaining on adaxial but not abaxial leaf surfaces *(Figure 1c)*. Ultra violet and simulated tropical solar radiation were both damaging to conidia, although capilliconidia were significantly more tolerant to radiation than primary conidia *(Figures 1d* and *1e)*. Neither primary conidia nor secondary capilliconidia were affected by 1 h of exposure to simulated tropical solar radiation, but 4 h exposure significantly reduced the number of infective conidia of each type remaining. Eight hours of exposure had no further affect on secondary capilliconidia but reduced the number of infective primary conidia to 13% of the original number *(Figure 1e)*.

Table 1. Evaluation of the sex pheromone trap

Parameter	Result of evaluation
Periodicity of male response to pheromone	Mean day-time catch= 22.8 (±2.2) Mean night-time catch= 29.3(±10.7) Male moths attracted to synthetic sex pheromone lures at all times of the day ($f_{1,6} = 0.27$, $p > 0.05$).
Time spent by male moths inside the inoculation chamber of the trap	88 (±1) seconds; (n=106)
Time repuired in inoculation chamber to ensure inoculation:	
Inoculum above pheromone lure	LT_{50} = 303 (126–630) seconds
Inoculum above and below pheromone lure	LT_{50}< 60 seconds
Field longevity of male moths	4.9(±0.3) days
Time for fungus to kill male moths in the field	3–4 days
Time for cadaver to produce conidia in the field	4–5 days

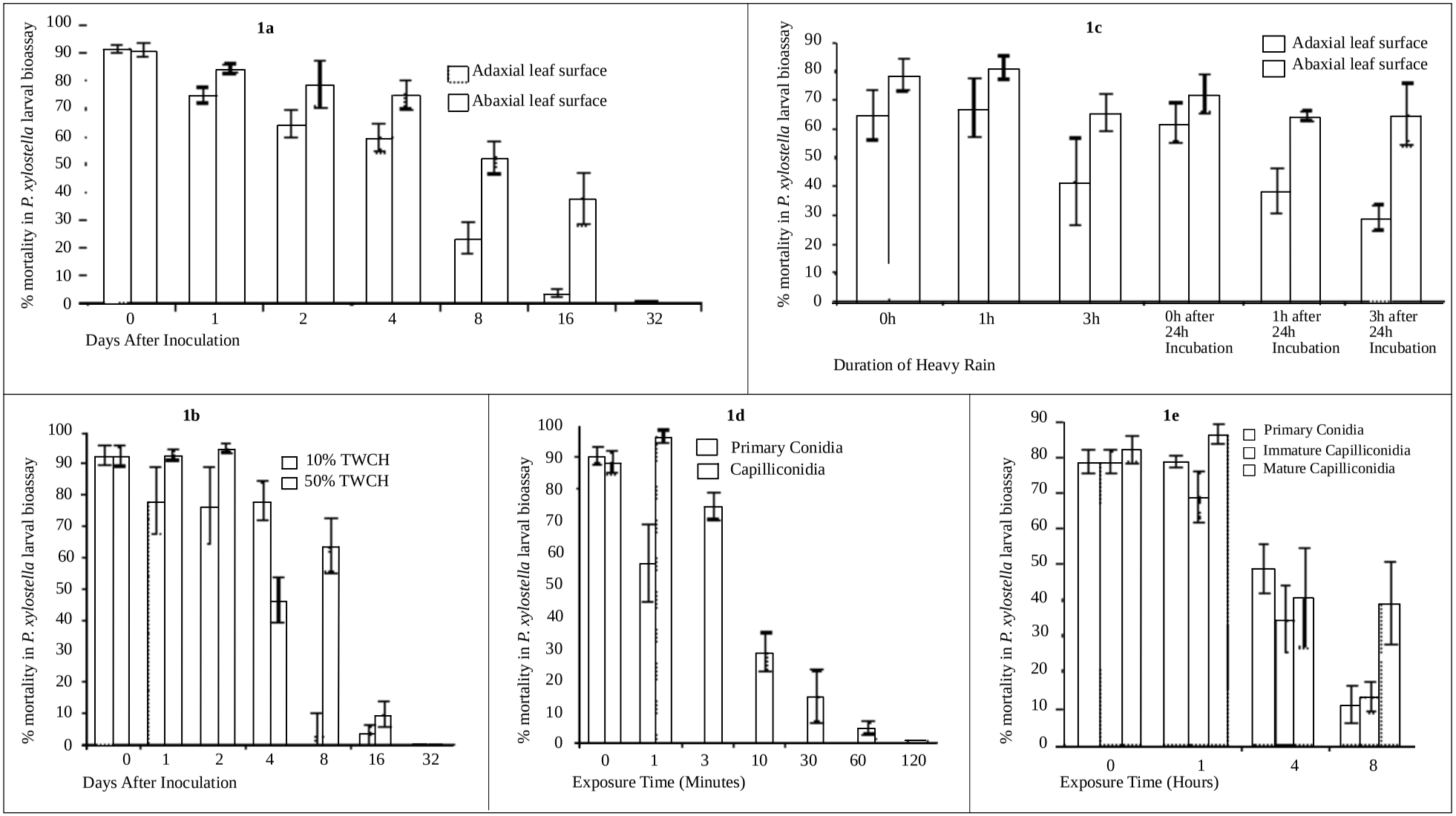


Figure 1. The effect of ***a)*** *fluctuating "field" temperatures* ***b)*** *soil moisture content* ***c)*** *heavy rainfall* ***d)*** *ultra violet radiation and* ***e)*** *simulated tropical sunlight on the persistence of* ***Zoophthora radicans*** *conidia*

Table 2. Interactions between *Zoophthora radicans* and two parasitoids of *Plutella xylostella*

	Adult (A)/larval (L) *P. xylostella*	Adult *D. semiclausum*	Adult *C. plutellae*
% Mortality induced in *Z. radicans* maximum challenge primary screen	100	90	0
Z. radicans leaf shower bioassay, estimated LC_{50} (conidia/mm^2)	A= 1.2(0.3–4.1) L= 2.3 (0.5–8.6)	163.4(43.8–1394)	–
Relative susceptibility to *Z. radicans* infection	A= 133 L= 70	1	
Proportion (95% CI) of larvae dying from *Z. radicans* infection when foraged upon by a parasitoid in the presence of a source of *Z. radicans* inoculum[1]	–	0.38 (0.25–0.47)	0.12 (0.06–0.25)
Evidence for parasitoid vectoring conidia to hosts	–	none	none
Distance (mm) moved by larvae foraged upon by parasitoid[2]	–	339.4 (±32.4)	236.1 (±50.4)
No. infective units entered by larvae[3]	–	39.1 (±3.8)	22.1(±4.2)

[1]When no parasitoids present the proportion of larvae from *Z. radicans* infection = 0.6 (0.08–0.29). *D. semiclausum* significantly increased the proportion of larvae dying from disease ($F_{2,27}$= 5.03, p <0.05).
[2]When no parasitoid present the distance (mm) moved by larvae = 68.2 (±27.2). This is significantly different from the distances moved when parasitoids were present, (t >3.36, df = 8; p <0.01 in each case). Larvae moved further in the presence of *D. semiclausum* than in the presence of *C. plutellae* (t >2.31, df = 8; p <0.05)
[3]Infective unit = area over which conidia are discharged= 100 mm^2. When no parasitoid present the number of infective units entered= 7.5 (±2.5). This is significantly different from the entered when *D. semiclausum* or *C. plutellae* were present, (t >3.36, df = 8; p <0.01 and t >2.31, df= 8; p <0.05, respectively). Larvae moved into are infective units in the presence of *D. semiclausum* than in the presence of *C. plutellae* (t >2.31, df= 8; p <0.05).

Interactions between the pathogen and important parasitoids of the diamondback moth

Cotesia plutellae was not susceptible to *Z. radicans* infection. The susceptibility of adult *D. semiclausum* to *Z. radicans* infection was 70- and 133- fold less than the susceptibility of *P. xylostella* larvae and adults, respectively *(Table 2)*. Neither species of parasitoid was shown to vector *Z. radicans* conidia directly from sporulating cadavers to the susceptible larvae upon which they foraged. The presence of a foraging *D.semiclausum* female enhanced the level of *Z. radicans* infection in *P. xylostella* larvae feeding in proximity to a source of *Z. radicans* inoculum *(Table 2)*. Video analysis of the movement of *P. xylostella* larvae in the presence and absence of the parasitoids indicated that the increased levels of fungal infection in the presence of *D. semiclausum* could be accounted for by the different types of movement (*i.e.* total distance moved and number of infective units entered) exhibited by larvae in the presence of this parasitoid when compared to the movement exhibited by larvae in the presence of *C. plutellae* or when no parasitoid was present *(Table 2)*.

Discussion

The mean time that male moths spent in the inoculation chamber of the pheromone trap in the field was compatible with the time required under a shower of primary conidia to have a 50% chance of acquiring a lethal infection of *Z. radicans*. Increasing the amount of inoculum from one plate of sporulating mycelia to two plates (one held above and one held below the pheromone lure) dramatically reduced the time required to acquire a lethal infection to less than 60 seconds. Under these conditions a male remaining in the trap for the mean duration measured in the field is almost certain to become infected. Infection rates may be enhanced further by increasing the amount of inoculum in the inoculation chamber, prolonging the male visiting time in the inoculation chamber (by modification of the baffle system of the trap or by incorporation of an arrestant in the inoculation chamber) and by increasing the intimacy between the moths and the inoculum (by incorporation of the synthetic pheromone into an agar medium beneath the sporulating mycelia). Although male moths inoculated with *Z. radicans* conidia in the trap are capable of vectoring conidia to other susceptible individuals, transmission rates are low. However, moths leaving the trap will themselves succumb to infection 3–3.5 days later and establish a potential focus of infection when they die from mycosis. In this way fresh conidia are made available for further infection of the pest population for far longer than with conventional means of application.

Primary conidia persisted for 24–72 h on foliar and soil surfaces in the field but most conidia lost viability within 24 h. It is likely that some primary conidia inoculated onto the leaf/soil surface germinated during the night to form capilliconidia, however, any capilliconidia which did form also failed to retain viability for much longer than 24 h. The field temperatures in the Cameron Highlands are comparable to the optimal temperatures reported for *Z. radicans* development (Milner and Lutton, 1983). In the laboratory, at alternating temperatures analogous

to those in the field study, conidia remained viable on foliage for up to 16 days after inoculation. However, there was a significant difference between the level of persistence on the adaxial and the abaxial surfaces, conidia losing viability more rapidly on the adaxial than on the abaxial surface. Relative humidity is often higher on the abaxial leaf surface than on the adaxial leaf surface (Oke, 1987) and persistence of *Z. radicans* conidia is known to be enhanced in conditions of high humidity (Uziel and Kenneth, 1991). It is therefore possible that higher RH on abaxial leaf surfaces resulted in the greater persistence of viable conidia in these locations on the plant. Little is known about the persistence of entomophthoralean fungi on the surface of soil. The demonstrated greater persistence of conidia on clay loam soil with a high moisture content in the laboratory is in accord with previous reports that entomophthoralean fungi retain viability for extended periods in conditions of high humidity. In the Cameron Highlands cabbage fields are frequently fertilised by the addition of chicken manure to the soil, which increases its water holding capacity. However, any increase in the persistence of *Z. radicans* conidia that this may facilitate is probably negated by the effects of the extra microflora contained in the manure. In laboratory experiments viable conidia persisted for longer on the surface of soil than on foliage *(Figures 1a* and *1b)* probably as a result of the differential effects of antimicrobial compounds known to be produced by foliage.

Entomophthoralean fungi are known to have pre-formed mucous which attaches them to the surfaces on which they land (Eilenberg *et al.*, 1986). Simulated rainfall did not affect the number of viable conidia remaining on the protected abaxial leaf surfaces. It did, however, reduce the number of viable conidia on the unprotected adaxial surface. Incubation of primary conidia on the surface of leaves under conditions of high relative humidity leads to germination and the development of capilliconidia (Pell *et al.*, 1993b). Capilliconidia are held away from the surface of the substrate by capillary conidiophores (Balazy, 1993). When the effect of rain capilliconidia was assessed the impact was found to be far greater than on primary conidia, even 1 h exposure to heavy rain causing a significant decrease in the number of viable conidia remaining on the adaxial leaf surface *(Figure 1c)*. We suggest that the impaction of rain droplets on capilliconidia which were still attached to conidiophores caused a greater amount of wash-off than it did to primary conidia which were firmly attached to the leaf surface. The vibration caused by the impaction of rain droplets on the adaxial surfaces was, apparently, insufficient to dislodge the capilliconidia from the conidiophores on the abaxial surfaces or, if they were dislodged, they were unable to break through the boundary layer and, after dislodgement fell back onto the leaf surface. Prolonged heavy rainfall reduced the number of viable conidia on the adaxial leaf surface. It is possible that the extra bombardment with water droplets and/ or the extra run off from the leaf surface was sufficient to loosen the attachment of the conidia allowing their dislodgement from the leaf surface. Alternatively, conidia may have been physically damaged by the water droplets landing upon them.

The preliminary ultra violet bioassay was designed to examine any differences in tolerance to ultra violet radiation between primary conidia and capilliconidia. Primary conidia were extremely susceptible to the intensity and wavelength of ultra violet radiation tested, becoming inviable after only 3 minutes exposure, while more than 60 minutes exposure was required to render all capilliconidia inviable. It is possible that capilliconidia, which are darker than primary conidia, possess melanin within their walls. In other systems the pigment absorbs ultra violet light and thereby reduces the rates of photoactivation reactions leading to the production of hydrogen peroxide within the cells of the conidia. One hour of exposure to simulated tropical sunlight had no effect on the number of viable primary conidia, immature secondary capilliconidia or mature capilliconidia remaining on irradiated leaf discs. However, 4 h exposure reduced the number of viable conidia of all types. Exposure for 8 h further reduced the number of viable primary conidia but had no further effect on either type of capilliconidia. Here there is a similarity with the results of the ultra violet exposure experiment in that capilliconidia are more tolerant to simulated tropical sunlight than primary conidia.

It is very difficult to separate the field effects of solar radiation, rainfall/ humidity and temperature. The laboratory work accompanying the field experiments leads to the conclusion that solar radiation is the major factor affecting the viability of *Z. radicans* conidia in the field in the Cameron Highlands of Malaysia. Despite being significantly more tolerant to solar radiation than primary conidia the vast majority of capilliconidia lose viability within 24 h of field exposure. The pheromone trap based system for delivering *Z. radicans* conidia directly to *P. xylostella* provides some protection from solar radiation for sources of inoculum as they are encased within the perspex of the trap before they are picked up by male moths and transported to the crop. However, when infected male moths succumb to the disease and primary conidia are actively discharged from the cadaver they are only likely to remain viable for a short period of time outside of the host.

The extremely low persistence rates which have been demonstrated for conidia lead us to consider just how *Z. radicans* may persist in the field. Generally, *Z. radicans* is considered to persist outside the host via the development of resting spores (Glare & Milner, 1991). However, NW250R is not known to produce resting spores but does cause *Z. radicans* epizootics in *P. xylostella* populations in the Cameron Highlands. Although the persistence of conidia may be enhanced by the protection conferred by shaded sites within the crop canopy, shading effects are likely to be limited

in conditions of bright sunlight as almost all harmful shortwave radiation (< 400nm) passes directly through leaves (Montieth, 1965). A more likely source of protection from the damaging effects of solar radiation is the insect host itself. Hyphal bodies developing within the haemocoel of the living insect and mycelia growing within the cadaver are shielded from solar radiation by the body of the host insect. The role of the host in providing the pathogen with its major source of environmental protection is currently under investigation.

Individual strains of *Z. radicans* are considered to be evolutionarily adapted to infect only taxonomically related insect hosts (Papierok *et al.*, 1984). The susceptibility of *D. semiclausum* (although approximately 100- fold less than that of the *P. xylostella* target) is therefore surprising and raises the question of the safety implications of initiating *Z. radicans* epizootics within *P. xylostella* populations which are, at times, regulated by *D. semiclausum*. Species of entomopathogenic fungi with wide host ranges in the laboratory are frequently more specific under field conditions and, as the control strategy under development maintains the field levels of inoculum as low as possible by directly targeting the pest, the chances of foraging *D. semiclausum* adults encountering high levels of inoculum are minimised. Furthermore, certain insect species may be easily infected in the laboratory by fungi which are not known to attack them in nature (Goettel, 1994). Despite examining cabbage fields supporting epizootics of *Z. radicans* in a *P. xylostella* population which also harboured *D. semiclausum*, *Z. radicans* infected *D. semiclausum* individuals were never found in the field in the Cameron Highlands. In addition, there are no reports of adverse effects of *Z. radicans* on *D. semiclausum* populations in the literature, despite close monitoring of field populations of the parasitoid and extensive studies of its ecology in Malaysia and other South East Asian countries over the past decade (Talekar and Griggs, 1986; Talekar, 1992). With the added knowledge that no *Z. radicans* infection in *D. semiclausum* was observed in any of the vectoring/ interaction experiments reported it is concluded that the laboratory demonstrated physiological susceptibility of *D. semiclausum* to this particular strain of *Z. radicans* is unlikely to translate into field, ecological susceptibility. Finally, the fungus was originally isolated from *P. xylostella* in the Cameron Highlands of Malaysia and, as Goettel (1994) contends it is unlikely that artificial augmentation of the inoculum of an indigenous fungus will cause permanent ecological damage in a given region.

Neither *D. semiclausum* nor *C. plutellae* was demonstrated to mechanically vector *Z. radicans* conidia from infected cadavers to larvae upon which they were foraging. Although *D. semiclausum* is not able to vector *Z. radicans* infection to *P. xylostella* larvae, the presence of foraging individuals increases the larval movement to such an extent that infection levels are enhanced. Thus, the presence of foraging *D. semiclausum* within populations of *P. xylostella* which harbour *Z. radicans* can potentially enhance fungal infection levels in the pest population and this may increase overall control levels in the field.

Acknowledgements

MJF was funded by a grant from the Leverhulme Trust and is currently funded by a grant from the ODA. One of the field visits to Malaysia was funded by the Royal Society. The Deputy Director General of MARDI is thanked for his permission to allow field work to be carried out at the Cameron Highlands Field Station. Particular thanks are extended to Mdm Ong Pek Choo, Dr Syed Abdul Rahman and Dr A. Sivapragasam. IACR-Rothamsted receives grant aided support from the Biotechnology and Biological Research Council of Great Britain.

References

Aruta, M. C., Carillo, L. R. & Gonzalze, M. S. (1974). Determinacion para Chile de hongos entomopatogenos del genero Entomophthora. *Agro Sur* ***2:*** 62–70. (In Spanish, English summary)

Balazy, S. (1993). Flora of Poland, Fungi vol. XXIV Entomophthorales. Polish Academy of Sciences, Krakov. 356 pp.

Furlong, M. J., Pell, J. K., Ong, P. C. and Syed, A. R. (1995). Field and laboratory evaluation of a sex pheromone trap for the autodissemination of the fungal entomopathogen *Zoophthora radicans* by the diamondback moth, *Plutella xylostella. Bulletin of Entomological Research* ***85:*** 331–337

Furlong, M. J. and Pell, J. K. (1996). Interactions between the fungal entomopathogen *Zoophthora radicans* Brefeld (Entomophthorales) and two hymenopteran parasitoids attacking the diamondback moth, *Plutella xylostella* L. *Journal of Invertebrate Pathology* ***68:*** 15–21

Furlong, M. J. and Pell, J. K. (1996). Influence of environmental factors on the persistence of *Zoophthora radicans* conidia. *Submitted to Journal of Invertebrate Pathology*

Eilenberg, J., Bresciani, J. and Latge, J-P. (1986). Ultrastructural Studies of Primary Spore Formation and Discharge in the Genus *Entomophthora*. *Journal of Invertebrate Pathology* ***48:*** 318–324

Glare, T. R. and Milner, R. J. (1991). Ecology of Entomopathogenic Fungi. In *Handbook of Applied Mycology, Volume 2: Humans, Animals and Insects* (eds. D. K. Arora, L. Ajello and K. G. Mukerji) pp. 547–612. Marcel Dekker Inc., New York

Goettle, M. S. (1994). Host range and specificity in relation to safety of exotic fungi. *VI th International Colloquium on Invertebrate Pathology and Microbial Control, XXVII th Annual Meeting of the Society for Invertebrate Pathology*, Montpellier, France 28 August – 2 September, 1994, pp. 325–329

Kanervo, V. (1946). Sporadic observations concerning diseases in certain species of insects 3. Diseases attacking *Plutella maculipennis* Curt. *Suomen Hyonteistieteellinen Aikakauskija* 12: 143–153.(In Finnish, English summary)

Milner, R. J. and Lutton G. G. (1983). Effect of temperature on *Zoophthora radicans* (Brefeld) Batko: an introduced microbial control agent of the spotted alfalfa aphid, *Therioaphis trifolii* (Monell) f. *maculata*. *Journal Australian Entomological Society* ***22:*** 167–173

Montient, J. L. (1965). Radiation and crops. *Exp. Agric. Rev.* ***1:*** 241–251

Oke, T.R. (1987). Boundary Layer Climates. Routledge. 435 pp.

Ooi, P. A. C. (1981). Microbial control of the diamondback moth in Cameron Highlands, Malaysia. *Malays. Appl. Biol.* ***10(1):*** 49–56

Papiekok, B., Valadao, B., Torres, L. & Arnault, M. (1984). Contribution to the study of the specificity of the entomophathogenic fungus *Zoophthora radicans* (Zygomycetes, Entomophthorales) (In French, English summary). *Entomophaga* ***29:*** 109–119.

Pell, J. K., Macaulty, E. D. M. & Wilding, N. (1993a). A Pheromone Trap for Dispersal of the Pathogen *Zoophthora radicans* Brefeld. (Zygomycetes: Entomophthorales) amongst Populations of the Diamondback moth, *Plutella xylostella* L. (Lepidoptera; Yponomeutidae). *Biocontrol Science and Technology* ***3:*** 315–320

Pell, J.,K., Wilding, N., Player, A. L. & Clark S. J. (1993b). Selection of an Isolate of *Zoophthora radicans* (Zygomycetes: Entomophthorales) for Biocontrol of the Diamondback Moth *Plutella xylostella* L. (Lepidoptera: Yponomeutidae). *Journal of Invertebrate Pathology* ***61:*** 75–80

Riethemer, G. W., Rombach, M. C. & Kranz, J. (1992). Epizootics of *Pandora blunkii* and *Zoophthora radicans* (Entomophthoraceae: Zygomycotina) in diamondback moth populations in the Philippines. In *Diamondback Moth and Other Crucifer Pests, Proceedings of the Second International Workshop, Tainan, Taiwan* (ed. N. S. Talekar) pp. 193–199. AVRDC

Talekar, N. S. (1992). *Diamondback Moth and Other Crucifer Pests, Proceedings of the Second International Workshop, Tainan, Taiwan*. 603 pp. AVRDC

Talekar, N. S. and Griggs, T. D. (1986). *Diamondback Moth Management, Proceedings of the First International Workshop, Tainan, Taiwan*. 471 pp. AVRDC

Ullyet, G. C. & Schonken, D. B. (1940). A Fungus Disease of *Plutella maculipennis*, Curt.,in South Africa, with Notes on the Use of Entomogenous Fungi in Insect Control. *Science Bulletin, Department of Agriculture and Forestry, Union of South Africa* ***218:*** 1–24

Uziel, U. and Kenneth, R.G. (1991). Survival of Primary Conidia and Capilliconidia at Different Humidities in *Erynia* (subgen. *Zoophthora*) spp. and in *Neozygites fresenii* (Zygomycotina: Entomophthorales), with Special Emphasis on *Erynia radicans*. *Journal of Invertebrate Pathology* ***58:*** 118–126

The feasibility of using sterile insect technique for the control of diamondback moth on cabbage in Cameron Highlands

Dzolkhifli Omar[1] and Mohd Jusoh Mamat[2]

[1]*Department of Plant Protection, Faculty of Agriculture Universiti Pertanian Malaysia*
[2]*Strategic, Environment and Natural Resources Center MARDI 43400 Serdang, Selangor*

Abstract

The feasibility of using the sterile insect technique to reduce the population of the diamondback moth (DBM), *Plutella xylostella* was investigated. The partial sterility or inherited sterility was selected as opposed to complete sterility. The fecundity, sterility and progeny development of parental crosses and certain F-1 backcrosses (progeny of irradiated males) were first studied in the laboratory following exposure of the pupae of DBM to substerilizing doses of gamma radiation. Doses above 200 Gy greatly affected the development of larvae in parental crosses of irradiated females with normal males and of irradiated males with irradiated females, as no pupation was observed. The doses of between 150 and 200 Gy appeared to be suitable for inherited sterility of the DBM.

Mating competitiveness studies for different ratios of irradiated male, normal male and normal female was conducted in the laboratory. The pupae were irradiated at 200 Gy. The ratio of 1:10:1 (normal male : irradiated male : normal female) and 1:20:1 showed no significant difference with 0:1:1, but showed significant difference with control (1:0:1) in terms of percentage egg hatch. The percentage of egg hatch for control was 81.4% while for 0:1:1 was 25.1%.

The field cage study was conducted in Cameron Highlands by releasing various ratio of irradiated DBM pupae to normal pupae. The pupae were again irradiated at 200 Gy. Based on the total number of larvae for 3 successive generations, the ratio of 10 irradiated :1 unirradiated significantly reduced the population of the DBM. No larvae was observed after 3 generations. At the ratio of 1:1, the number of larvae were reduced by 56% after 3 generations. The implication and logistic of the F-1 sterility technique in relation to the DBM control were discussed

Key words: inherited sterility, *Plutella xylostella*, mating competitiveness.

Introduction

The diamondback moth (DBM), *Plutella xylostella*, was first recorded in Frasers Hill, Pahang, in 1925 and became a major pest of cabbage in the Cameron Highlands in 1934 (Corbett and Pagden, 1941). Since then, it has been widely recognized as a serious pest of crucifers in both highland and lowland areas. Total loss could reach a level of 70–90% if no control measure was taken. Even though insecticides have been successful to some extent in controlling this insect, heavy reliance on, and indiscriminate use of, insecticides have caused the DBM to become resistant to most of the insecticides available on the market (Fauziah *et al*., 1990; Syed, 1990). The need for other control methods has risen following the failure of insecticides to control this pest. One such method has been the use of sterile or partially sterile insect technique.

The technique of partial sterility or inherited sterility is preferred for the control of lepidopterous pests as complete sterilization tends to induce physiological disturbances such as reduction of mating competitiveness, lack of sperm transfer, etc. The progeny of the irradiated parents at substerilizing doses have also been shown to be more sterile than the parents. The potential of inherited sterility in pest control was demonstrated by Knipling (1970) through a mathematical model. Numerous studies have been conducted on lepidopterous pests such as *Helicoverpa zea*, *Spodoptera frugiperda* and *Trichoplusia ni*. Inherited sterility was reviewed extensively by LaChance (1985). The DBM has been suggested as a potential candidate for inherited sterility.

Effect of substerilization doses of radiation on DBM

Diamondback moths were collected from Serdang in Selangor and cultured on Chinese mustard (sawi) leaves in the laboratory at 27 ± 2 °C and 80 ± 15% relative humidity. The larvae were given fresh leaves daily while adults were fed with 5% honey solution soaked in cotton wool. The pupae were collected and irradiated at 1 day before emergence by using a gamma cell (^{60}Co) irradiator that had a dose rate of 60 Gray (Gy)/min. The pupae were irradiated at 100, 150, 200 and 250 Gy. The males were then separated from the females. The emerging males (m) and females (f) were paired for the following parental crosses (I, irradiated; N, normal; A, progeny of the cross $I_m \times I_f$): $I_m \times I_f$, $I_m \times N_f(A)$, $N_m \times I_f$, $N_m \times N_f$. The male and female adults emerging from the parental cross of $I_m \times N_f(A)$ were then paired with normal female and male adults, respectively, For F_1 backcrosses and designated as follows: $A_m \times N_f$, $A_f \times N_m$ and the development of the F_2 generation was then studied.

Five pairs of DBMs were placed in a 2.5 L plastic cylinder for mating and oviposition. The adults were provided with 5% honey solution. A fresh sawi leaf was placed in the plastic cylinder for oviposition. After 2 days, the leaf was removed and replaced with another fresh leaf for a further 2 days. The total number of eggs deposited on both leaves was counted to assess the relative fecundity of females from different crosses. The number of eggs hatched was recorded and fresh leaves were supplied daily to the larvae. The larvae were then allowed to complete their development. The percentages of fourth instar larvae and pupae obtained from the eggs hatched were used for a statistical analysis.

The development of DBM obtained from the parental crosses was found to be dependent on dose. A dose above 200 Gy greatly affected oviposition and egg hatch to larvae *(Tables 1* and *2)*. At doses of 200 and 250 Gy, parental crosses of I_m x I_f and N_m x I_f failed to produce pupae. In those crosses the number of eggs deposited was also greatly reduced compared with the lower doses and the control ($p<0.05$), indicating that a dose above 200 Gy reduced female fecundity. However, the numbers of eggs deposited after doses of 100 and 150 Gy were not significantly different when compared with the control N_m x I_f crosses *(Table 2)*. The proportion of the egg hatch was significantly reduced, indicating that these doses caused female sterility.

Parental crosses of irradiated males with normal females showed no apparent loss of fecundity of females but significantly reduced the egg hatch *(Table 2)*. However, the percentages of eggs hatch and larvae surviving to pupae from these crosses appeared to be higher compared with the other parental crosses. The F_1 backcross of either male or female from I_m x N_f showed no significant decrease in fecundity but significant reductions in egg hatch of F_2 generation larvae and pupae compared with control crosses ($p<0.05$) *(Table 2)*.

It appears from the results of parental and F_1 crosses that the dose of 100 Gy may not be suitable for inherited sterility because of the significantly lower sterility demonstrated. Results on the female progeny from A_m x N_f backcrossed with normal males showed almost no difference in the percentage of eggs hatched ($\approx 67\%$) compared with the control. However, the F_2 male progeny from the same cross backcrossed with normal females still exhibited sterility (hatchability of about 45%).

A dose of between 150 and 200 Gy should be used for further study in the development of the DBM inherited sterility programme. A dose of 250 Gy may not be suitable, since it caused deformities in 30% of the emerged adults. It should be noted that at a radiation dose of either 150 or 200 Gy, no increase in sterility in the backcross generation was observed. The dose of 100 Gy, however, has been shown to have greater inherited deleterious effects for the progeny of irradiated males of fall army worms (Carpenter *et al*., 1986). A similar dose was also used to sterilize corn earworms partially (Carpenter *et al*., 1987). It could be that the DBM may require a higher dose of radiation for inherited sterility as it has a relatively small size and short life cycle in the tropics. The strain used also has developed resistance to almost all the insecticides available on the market.

Table 1. Effect of doses of radiation on fecundity of parental crosses of irradiated and normal *P. xylostella* pupae

Radiation dose (Gy)	I_m x I_f*		N_m x I_f*		I_m x N_f*	
	Total number of eggs	Egg hatch (%)	Total number of eggs	Egg hatch (%)	Total number of eggs	Egg hatch (%)
Control	404a	79.4a	404a	79.4a	404ab	79.4a
100	231b	6.3b	350a	20.3	301b	58.9b
150	221b	2.6bc	388a	10.5c	571a	49.7c
200	47c	0.0c	26b	0.0d	469ab	39.6d
250	53c	0.0c	40b	0.0d	475a	40.3d

*Values in each columns having the same letter are not significantly different at $p<0.05$

Table 2. Effect of doses of radiation on the fecundity of F_1-generation backcross with normal male and female of *P. xylostella*

Radiation dose (Gy)	A_f x N_m*		A_m x N_f*	
	Total number of eggs	Egg hatch (%)	Total number of eggs	Egg hatch (%)
Control	404a	79.4a	404a	79.4a
100	340ab	35.9c	436a	51.7b
150	444ab	46.7bc	378a	37.0c
200	576a	49.7b	359a	50.9b
250	na	na	253a	35.2c

*Values in each columns having the same letter are not significantly different at $p<0.05$; na: not available

Mating Competitiveness

The pupae were irradiated at the doses of 150 and 200 Gy one day before emergence and separated between males and females. The unirradiated male, irradiated male and unirradiated female were placed in 2.5 L cylinder at the following ratios: 1:0:1, 0:1:1, 1:1:1, 1:5:1, 1:10:1 and 1:20:1. The adults were allowed to emerge and mate. Honey (5%) was made available to the adults. There were 4 replicates/dose/ratio. The number of eggs laid and percentage of egg hatch were recorded. Data were subjected to the statistical analysis.

In term of percentage egg hatch, all ratios show significant difference from the control *(Table 3)*. The ratio of 1:10:1 and 1:20:1 show similar percentage of egg hatch with the ratio of 0:1:1. This indicated that at those ratios, the irradiated males are able to compete with the unirradiated male for the female. The irradiation dose of 200 Gy gave lower percentage of egg hatch compared to 150 Gy. It appears that the dose of 200 Gy and release ratio of 1:10:1 would be the best combination to suppress the population of DBM in the field.

Field Cage Study

The study was conducted in Cameron Highlands. Four field cages, each measuring 3.66 m long x 1.83 m wide and 1.83 m high were planted with 20 cabbage plants, *Brassica oleraceae* (variety: K-Y Cross). Normal agronomic practices were followed. Irradiated (i) and non-irradiated (ui) laboratory-reared DBM were first irradiated at 200 Gy. In this study the male and female pupae of irradiated and non-irradiated were not separated. They were placed in the cages for the release 24 h following treatment. The number of pupae and ratios were 0i : 20ui, 20i : 20 ui, 200i : 20 ui and 400i : 20 ui. The number of larvae were counted over 20 plants for 1st generation (week 1–4), 2nd generation (week 5–8) and 3rd generation (week 9–12). The experiments were repeated 3 times over time (May–July, 1995, Sept–Nov, 1995 and Jan–March, 1996).

Table 3. The percentage of egg hatch at various ratios of unirradiated male and irradiated male to unirradiated female

Ratio	Radiation Doses (Gy)*	
	150	200
1:0:1	78.5a	81.4a
0:1:1:1	32.3d	25.1d
1:1:1	53.3b	43.6b
1:5:1	45.0c	35.4c
1:10:1	36.8d	22.7d
1:20:1	30.1d	20.7d

*Values in each columns having the same letter are not significantly different at $p<0.05$
Ratio = unirradiated male : irradiated male : unirradiated female

The ratio of 200i : 20 ui reduced the larval population up to 84.5% *(Table 4)* for the first generation and subsequently no larvae was observed on third generation. As the males and females were not separated in this trial, the actual ratio (assuming 50% male and 50% female in the population) would be 100 irradiated male : 100 irradiated female : 10 unirradiated male : 10 unirradiated female or 10:10:1:1. In the first study, the mating between irradiated female to normal male and irradiated male to irradiated female produced the eggs that failed to hatch *(Table 1)*. The result support the mating competitiveness study that showed the ratio of 1:10:1 at the dose of 200 Gy would be sufficient to reduce the population of DBM. The field cage study shows that the progeny of the irradiated parents were more sterile than the parents and the population was eliminated after 3rd generation.

In conclusion, the studies showed that technically, it is feasible to control *P. xylostella* using sterile insect technique in Cameron Highlands. However, economically it may not be feasible due to very high cost. At an average field population of 2 adults generated per plant/crop/generation, the total number of adults generated in the whole of Cameron Highlands per year is estimated about 20 million adults. At an effective release rate of 10 irradiated : 1 feral *P. xylostella*, a total of 200 million irradiated insect is required. At the cost of 3 cents/insect (excluding fixed cost), RM 6 million is required just to produce the insect. The cost does not include the cost of infrastructure for mass rearing and distributing and release of the irradiated insect in the cabbage growing areas. Nevertheless, the technique is available if other control methods fail to bring the diamondback moth under control.

Acknowledgement

This work was funded by IRPA, Ministry of Science and Technology, Malaysia, and the IAEA. The authors wish to thank the Nuclear Energy Unit for radiation

Table 4. Effect of release ratios of irradiated DBM pupae to unirradiated pupae on 3 successive generations of DBM larval populations on cabbages planted under field cages

Treatment ratio	F-1		F-2		F-3	
	Number of larvae	% larval reduction	Number of larvae	% larval reduction	Number of larvae	% larval reduction
0i:20ui	45.3 ± 23.5	0.0	63.6 ± 50.5	0.0	23.3 ± 17.2	0.0
20i:20ui	27.0±4.0	40.4	21.7 ± 21.7	65.9	10.3 ± 3.1	55.8
200i:20ui	7.0 ± 3.5	84.5	2.7 ± 4.6	95.8	0.0	100
400i:20ui	3.7 ± 2.1	91.8	0.7 ± 1.2	98.9	0.0	100

facilities and Siti Aishah Johan, Mohamad Shah Abdullah, Ahmad Basir and Abu Zarim for technical assistance.

References

Carpenter, J.E., Young, J.R., Sparks, A.N. (1986). Fall armyworm (Lepidoptera: Noctuidae): Comparison of inherited deleterious effects in progeny from irradiated males and females. *J. Econ. Entomol.* ***79:*** 46–49.

Carpenter, J.E., Young, J.R., Sparks, A.N., Cromroy, H.L., Chowdhury, M.A. (1987). Corn earworm (Lepidoptera: Noctuidae): Effects of substerilizing doses of radiation and inherited sterility on reproduction. *J. Econ. Entomol.* ***80:*** 483–489.

Corbett, G.H., Pagden, H.T. (1941). A review of some recent entomological investigations and observations, *Malays. Agric. J.* ***29:*** 347–375.

Fauziah, I., Dzolkhifli, O., Wright, D.J. (1990). Resistance to acylurea compounds in diamondback moth. *In:* Management of Diamondback Moth and other Crucifer Pests (Proc. 2nd Int. Workshop, Taiwan) Talekar, N.S., Ed.), Asian Vegetable Research and Development Center, Taiwan. p. 391–402.

Knipling, E.F. (1970). Suppression of pest Lepidoptera by releasing partially sterile males: *A theoretical appraisal, Bioscience* ***20:*** 65–470.

LaChance, L.E. (1985). Genetic methods for the control of lepidopteran species: status and potential. ARS–28, USDA, Washington, DC.

Syed, A.R. (1990) Insecticide resistance in diamondback moth. *In:* Management of Diamondback Moth and other Crucifer Pests (Proc. 2nd Int. Workshop, Taiwan) Talekar, N.S., Ed.), Asian Vegetable Research and Development Center, Taiwan. 437–442.

Antifeedant activity of active fractions from a tropical plant, *Andrographis paniculata* Nees against the diamondback moth

Wawan Hermawan, Shuhei Nakajima, Ritsuko Tsukuda, Kenji Fujisaki and Fusao Nakasuji

Faculty of Agriculture,Okayama University,Tsushima, Okayama 700, Japan

Abstract

Guided by bioassay test results, we have isolated an active compound from the hexane layer of the extract of *Andrographis paniculata* and it was identified as 14-deoxyandrographolide. Although this compound has been known as a medicine in Pharmacology, its antifeedant activity against insects has never been reported yet. The minimum effective dose (MED = 100µg/leaf disc) on the isolated compound in suppressing the feeding of DBM larva however, is less potent than the hexane layer (MED = 30µg/leaf disc) and the fraction 5–2 (MED = 50µg/leaf disc). This result suggests that substance(s) other than 14-deoxyandrographolide present in the hexane layer may also be contributing towards feeding suppression against the diamondback moth larva.

Key words: *Andrographis paniculata, Plutella xylostella,* antifeedant 14-deoxyandrographolide.

Introduction

Andrographis paniculata is a herb endogenous in southeast Asia, China and India. It is widely used in traditional remedies and folkloric medicine to cure a variety of human illness. The increasing efforts to develop environmentally safe pest control methods has inspired us to study the potential of this plant to control the diamondback moth (DBM) *Plutella xylostella* L., which is considered as the most serious insect pest of cruciferous crops in many countries.

In our previous study, we reported that extracts from *A. paniculata* have antifeedant properties against the first and fourth instar larvae of DBM (Hermawan *et al.,* 1993; 1994) and antioviposition properties against the female adults (Hermawan *et al.,* 1994). Guided by bioassay tests, our present endeavor is focused on the isolation and identification of the active compound.

Materials and Methods

Plant Material

Fresh plant material was collected from central Java, Indonesia on April 1995. It was subsequently dried, cut into small pieces and packed before being brought to the Entomology Laboratory in Okayama University, Japan.

Test Insect

The initial population of the DBM was collected as larvae from a cabbage field located inside the campus of Okayama University. The collected insects were reared following an established procedure using cabbage leaves as feed under 25 °C temperature and 16L:8D photoperiod conditions.

Extraction and Purification

The dried powder obtained from the main stems, branches and leaves of *A. paniculata* (5.36 kg) were soaked in methanol three times. The resulting extract was partitioned using hexane, EtOAc, BuOH and water, successively. Each layer was concentrated, weighed and diluted to various doses (15µg to 2000µg) with 75% methanol in water. The hexane layer (2.0 g) was subjected to an open column chromatography (3 cm x 60 cm; silica gel 60), and eluted stepwise with 0, 5, 10, 30, 50 and 100% EtOAc in hexane and 100% methanol. Each of the seven fractions obtained from the hexane layer was evaporated *in vacuo,* weighed and diluted to various doses (12.5µg to 100µg) with 75% acetone in water to prepare the sample solutions for the bioassay test.

The fraction 5 (F.5) which was found as the most effective in suppressing the feeding of DBM larvae during the preliminary test, was further subjected to an open column chromatography (1 cm x 20 cm; silica gel 60) with solvent system hexane: EtOAc: acetone(7:2:1). Five fractions were obtained through this chromatographic process, and the test revealed that the fraction 5–2 at 50µg/leaf disc was the most potent antifeedant. Based on this result, the fraction 5–2 was further subjected to an open column chromatography with solvent system hexane: EtOAc (6:4) and then subjected through preparative TLC developed three times (silica gel 60 F_{524}) with solvent system chloroform:methanol(10:1), followed by reverse phase, C_{18} SEPPAK cartridge (methanol and water, 7:3) and finally purified through preparative TLC SiO_2–$AgNO_3$(10%) developed three times (hexane:EtOAc, 3:7) to afford the active compound (1.6 mg). The procedure of extraction and isolation is shown in *Figure 1.*

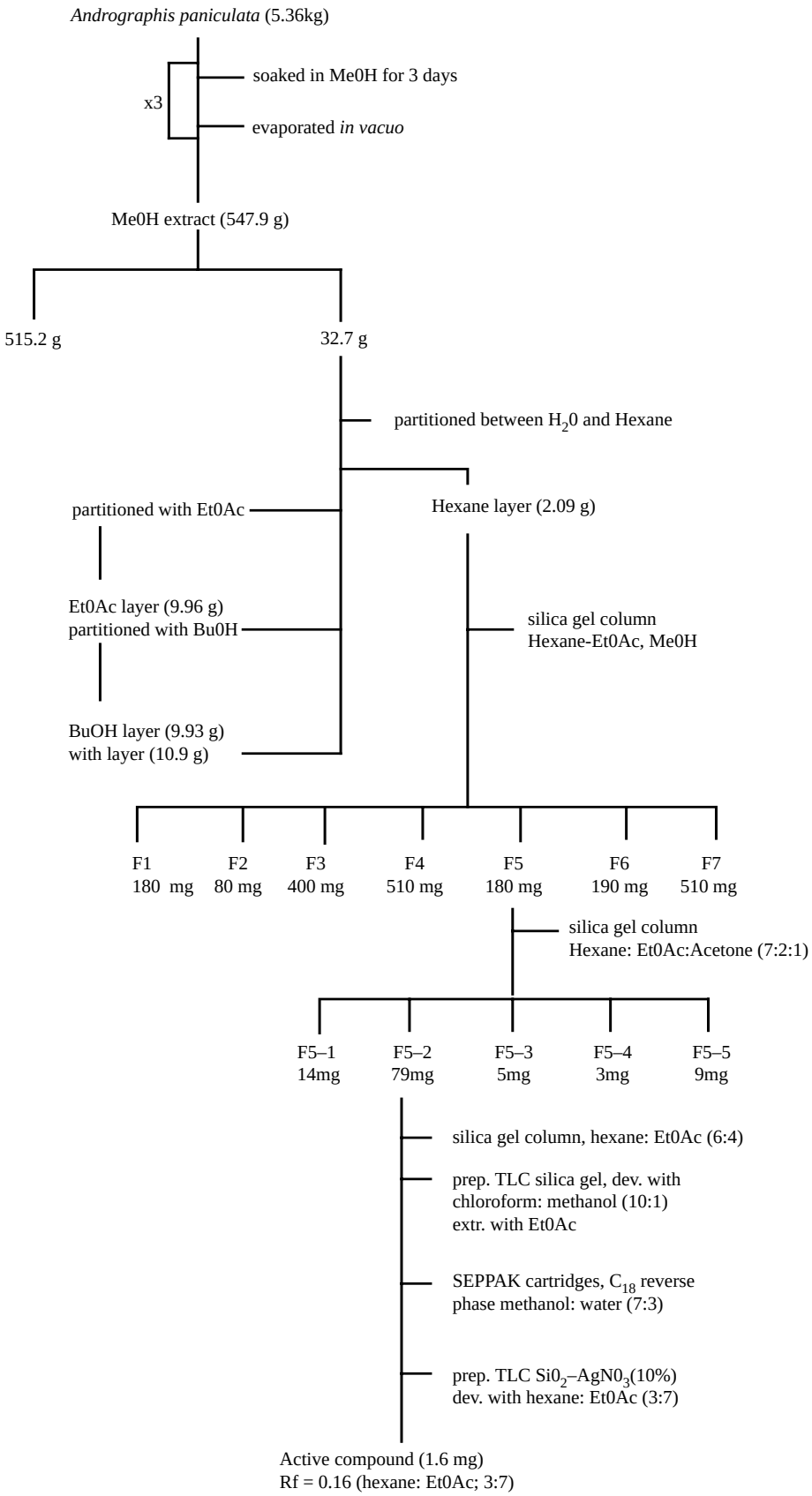


Figure 1. Schematic procedure used in isolating the active compound from the methanol extract of Andrographis paniculata

NMR Spectra were recorded on a Varian VXR 500 (500 and 125 MHz for ^{1}H and ^{13}C, respectively), IR on FT–IR 710 (Nicolet), MS on SX102A and automass 20 (JEOL) and UV on UV 3000 (Shimadzu) spectrometers.

Antifeedant test for DBM larvae

A no choice experiment was set up with 5 replicates for each test dose of a fraction. One untreated control was also set up. Fifty µl of each test fraction was applied to both surfaces of a leaf disc (2 cm in diam.) by using a micropipette and the excessive solution was air dried before placing it on wet filter paper fitted in an ice cream cup (6.5 cm in diam.). The leaf disc in the control was treated with the same volume of 75% acetone in water. Two 4th-instar larvae of DBM which have been starved for 3h were allowed to feed on each leaf disc for 24 h. The area of each leaf disc before and after feeding was measured using a digital leaf area meter(model LI-3050 A/4, USA). All tests were conducted under 25 °C temperature and 16L:8D photoperiod conditions.

Results

The crude extracts of *A. paniculata* have variable degrees of antifeedant potency against the 4th-instar larvae of DBM. At certain dose levels, larval feeding on leaf disc treated with the crude extracts were significantly lower($p<0.05$ by MANN-WHITNEY U-test) than the control treatment as shown by the degree of larval consumption on cabbage leaf discs during the 24h feeding period *(Figure 2)*. The hexane layer which was observed to be significantly different from the control at a considerably low dose (30µg/leaf disc) had the strongest antifeedant activity. Moreover, as the dose of this layer was increased, there was a corresponding increase in its potency. The EtOAc layer effectively reduced feeding at 250µg/leaf disc and higher doses. Significant antifeedant activity of the BuOH layer was observed at a relatively higher dose (1000µg/leaf disc) but feeding was tremendously reduced when it was treated at 2000µg/leaf disc. Regardless of dose, the water extract fairly deterred larval feeding.

The antifeeding potencies of the various fractions and the active compound obtained from the hexane layer through a series of fractionation and isolation works are presented in *Figure 3*. From a total of 7 fractions derived from this layer, fractions 4 to 7 were the most effective at 100µg/leaf disc. At a reduced dose (50µg/leaf disc) however, fraction 5 was the most effective. This finding led to further fractionation of hexane fraction 5 yielding 5 fractions (F5-1 to F5-2). When tested at 50µg/leaf disc, F5-2 was the most potent among these fractions. Several stages of silica gel chromatography were conducted and finally led to the isolation of an active compound (1.6mg) that significantly reduced larval feeding at 100µg/leaf disc.

The CI MS of the active compound showed a $[M+H]^{+}$ at *m/z* 335 revealing its molecular formula as $C_{20}H_{30}O_4$, which was consistent with the ^{1}H and ^{13}C NMR data. Two hydroxyl groups in the active compound were indicated by the strong IR absorption near 3300 cm^{-1} and by the formation of a diacetate. The IR spectrum also suggested that the compound has the moieties of an α,β-unsaturated γ-lactone(υmax 1754 and 1637 cm^{-1}) and an exocyclic methylene(υmax 1653 and 902 cm^{-1}). The olefinic proton signal in the ^{1}H NMR spectrum and the UV absorption (λmax 212 nm, ξ:10700) confirmed the presence of an endocyclic double bond in conjugation with the γ-lactone carbonyl. The chemical shift (δ 7.08) is similar to that found in analogous system. In addition, the ^{1}H NMR spectrum showed signals for the exocyclic methylene (δ 4.88 and 4.59, 1H each), two tertiary methyl groups (δ 1.23 and 0.63, 3H each), an oxymethine proton (δ 3.46, 1H) and two oxymethylenes (δ4.67, 2H ; δ 3.30 and 4.17, 1H each). These spectroscopic data strongly suggested that the active compound is a bicyclic diterpene with a 2-butenolide ring. Thus, on the basis of the structures of diterpenes isolated so far from this plant, the active compound was identified as 14-deoxyandrographolide

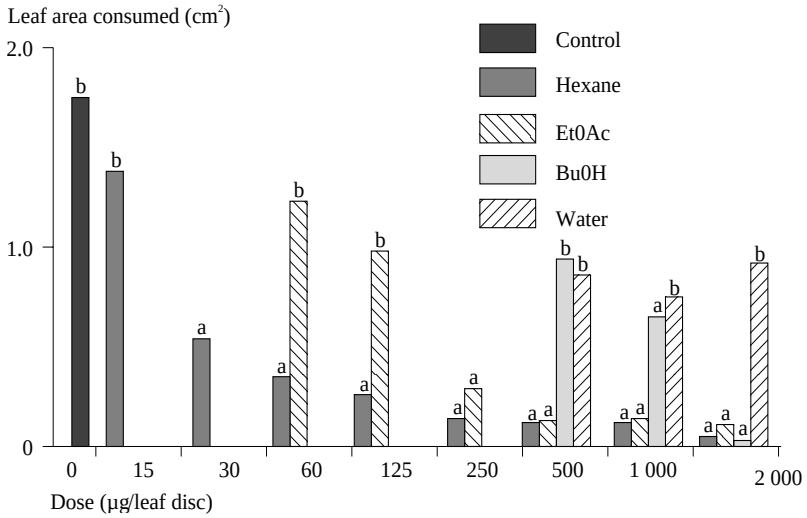


Figure 2. Mean of leaf area consumed by two 4th-instar larvae of DBM during the 24 h feeding period on cabbage leaf disc treated with different doses of each layer. Bars with the same letter are not significantly different at $p<0.05$ by MANN-WHITNEY U-test

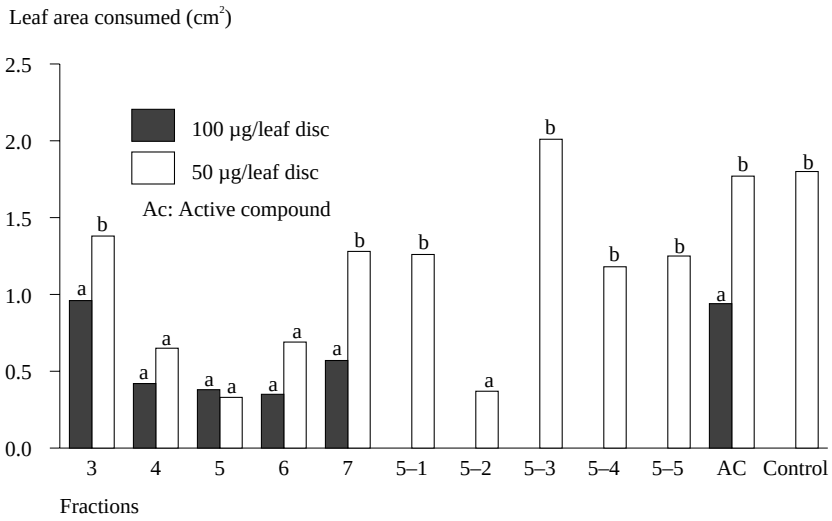


Figure 3. Means of leaf area consumed by two 4th-instar larvae of DBM during the 24h feeding period on cabbage leaf disc treated with the different fractions and the active compound (14-deoxyandrographolide) isolated from the hexane layer. Bars with the same letter are not significantly different at p <0.05 by MANN-WHITNEY U-test

(Figure 4), one of the *ent*-labdane diterpenoids, by comparison of the ^{1}H and ^{13}C NMR spectral data with that reported in literature (Balmain and Connolly, 1973; Jantan and Waterman, 1994; Fujita *et al.,* 1984 and Matsuda *et al.,* 1991).

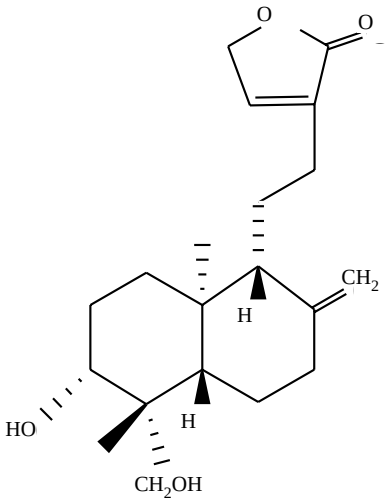


Figure 4. Structure of 14-deoxyandrographolide

Discussion

Recent research investigations on the herb *A. paniculata* are focused on evaluations for its medicinal values (Kusumoto *et al.,* 1992; Puri *et al.,* 1993 and Jalil *et al.,* 1996). Except for its nematicidal action (Bandhopadhyay *et al.,* 1986), studies on the bioactivity of *A. paniculata* against agricultural pests have been limited to the DBM (Hermawan *et al.,* 1993; Hermawan *et al.,* 1994), the azuki bean weevil *Callosobruchus chinensis* (Hermawan *et al.,* 1993) and the rice green leafhopper *Nephotettix cincticepts* (Widiarta, I. Nyoman, pers. comm.). In the previous study, Hermawan *et al.* (1994) reported that a crude extract from this plant had an antifeedant and antioviposition activities against the larva and moth of the DBM, respectively.

Guided by bioassay test results, we have isolated an active compound from the hexane layer of the extract of *A. paniculata* and it was identified as 14-deoxyandrographolide. The 14-deoxyandrographolide is a known compound but its antifeedant activity against the DBM has never been reported yet. Its minimum effective dose (MED) in suppressing the feeding of DBM larva is 100µg/leaf disc, less potent than the hexane layer (MED = 30µg/leaf disc) and the fraction 5–2 (MED = 50µg/leaf disc). It seems to show that other potentially deterrent compounds were lost during the fractionation and isolation that ensued. This result conforms to the observation of Puri *et al.* (1993), wherein the stimulation of both antigen specific and nonspecific immune response was higher with the EtOH extract than with the purified andrographolide, suggesting thereby that substance(s) other than andrographolide present in the extract may also be contributing towards immunostimulation. Similarly, Naumann and Isman (1995) noted that the crude suspensions of neem seeds *Azadirachta indica* had greater combined antilarval and antioviposition properties than the pure azadirachtin.

As mentioned previously, the crude extract from *A. paniculata* has also antioviposition activity against the azuki bean weevil and antifeedant activity against the rice green leafhopper. These results imply that the compound(s) from *A. paniculata* has nonspecific bioactivity, and it strongly suggests that compound can be used as a new control measure to contain a wide range of insect pests of agricultural crops.

The success in isolating the 14-deoxyandrographolide from the hexane layer of the extract of *A. paniculata* had stimulated us to further isolate and identify the other active principle(s) which may have a synergistic action in antifeedant properties against the larva of the DBM.

Acknowledgements

We would like to thank Prof. Naomichi Baba and Mr. Taro Takeda (Faculty of Agriculture, Okayama University) for their kind help in our experiment. We also thank Mr. Vic V. Casimero (Faculty of Agriculture, Okayama University) for valuable suggestion and critical reading of the manuscript.

References

Balmain, A. and J. D. Connoly (1973). Minor diterpenoid constituents of *Andrographis paniculata* Nees. *J. Chem. Soc. Perkin Trans.* ***1:*** 1 247.

Bandhopadhyay, K., S. K. Datta and N. C. Sukul (1986). A spectrophotometric estimation of andrographolide and examination of nematicidal activity of the crude extract of *Andrographis paniculata*. *Indian Drugs.* ***23:*** 510–512.

Fujita, T., R. Fujitani, Y. Takeda, Y. Takaishi, T. Yamada, M. Kido and I. Miura (1984). On the diterpenoids of *Andrographis paniculata:* x-ray crystallographic analysis of andrographolide and structure determination of new minor diterpenoids. *Chem. Pharm. Bull.* ***32(6):*** 2 117–2 125.

Hermawan, W., R. Tsukuda, K. Fujisaki, A. Kobayashi and F. Nakasuji (1993). Influence of crude extracts from a tropical plant, *Andrographis paniculata* (Acanthaceae), on suppression of feeding by the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae) and oviposition by the azuki bean weevil, *Callosobruchus chinensis* (Coleoptera: Bruchidae). *Appl. Entomol. Zool.* ***28(2):*** 251–254.

Hermawan, W., S. Kajiyama, R. Tsukuda, K. Fujisaki, A. Kobayashi and F. Nakasuji (1994). Antifeedant and antioviposition activities of the fractions of extract from a tropical plant, *Andrographis paniculata* (Acanthaceae), against the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae). *Appl. Entomol. Zool.* ***29:*** 533–538.

Jalil, J., N. I. Yusoff, Z. Zakaria and I. M. Said (1995). Phytochemical and bioactivity screening of aqueous extracts of several medicinal plants in Malaysia. *In Chemical prospecting in the Malaysian forest* (G. Ismail, M. Mohamed and L. Bin Din, Ed.). CTTC, University Malaysia Sarawak, pp. 1–7.

Jantan, I. and P. G. Waterman (1994). *ENT*-14b-hydroxy-8(17),12-labdadien-16, 15-olide-3b, 19-oxide: A diterpene from the aerial part of *Andrographis paniculata. Phytochemistry*. ***37:*** 1 477–1 479.

Kusumoto, I. T., I. Shidama, N. Kakiuchi, M. Hattori, T. Namba and S. Sutardjo (1992). Inhibitory effects of Indonesian plant extracts on reverstranscriptase of an RNA tumour virus (I). *Phytotherapy Research.* ***6:*** 241–244.

Matsuda, T., M. Kuroyanagi, S. Sugiyama and A. Ueno (1991). Studies on cell differentiation inducers of *Andrographis paniculata*. *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu.* ***33:*** 433–440.

Naumann, K. and M. B. Isman (1995). Evaluation of neem *Azadirachta indica* seed extracts and oils as oviposition deterrents to noctuid moths. *Entomologia Experimentalis et Applicata*. ***76:*** 115–120.

Puri, A., R. Saxena, R. P. Saxena. and K. C. Saxena (1993). Immunostimulant agents from *Andrographis paniculata. J. of Natural Products.* ***56:*** 995–999.

Trap crops for some cabbage pests of the Asia Pacific lowland tropics

R. Muniappan, T.S. Lali and Prem Singh
Agricultural Experiment Station University of Guam Mangilao, Guam 96923 U.S.A

Abstract

Trap cropping with Chinese cabbage cv. Tempest, radish cv. Minowase Summer Cross 3 and Indian mustard in cabbage field has significantly reduced the incidence of *Hellula undalis* (F.) *Crocidolomia pavonana* (F.) and *Halticus tibialis* (Reuter) on cabbage. There is a direct relationship to the infestation of *H. undalis* to the incidence of soft rot disease of cabbage in the field.

Key words: Trap crops, cabbage, *Hellula undalis, Crocidolomia pavonana, Halticus tibialis*, mustard, radish, Chinese cabbage.

Introduction

Cabbage (*Brassica oleracea* var. *capitata* L) on Guam is attacked by 10 arthropod pests (Muniappan and Marutani, 1992). Most of these pests also occur in the lowland tropics of the Asia Pacific region (Waterhouse and Norris, 1987, 1989; Waterhouse, 1993). Attempts to tackle these pests by environmentally safe, effective and economical methods lead to the research on trap cropping. Successful use of trap crops for control of rape blossom beetle, *Meligethes aeneus* on cauliflower in Finland (Hokkanen, 1989; Hokkanen *et al.,* 1986) and diamondback moth on cabbage in Bangalore, India (Srinivasan and Krishna Moorthy, 1991) have been reported in cruciferous crops. Hokkanen (1991) has reviewed use of trap crops for control of pests in different cropping systems. Muniappan and Marutani (1992) and Silva-Krott *et al.* (1995) have indicated the possibility of use of Chinese cabbage, radish and Indian mustard as trap crops in cabbage fields for control of cabbage webworm, *Hellula undalis* (F.) cabbage cluster caterpillar, *Crocidolomia pavonana* (F.) flea hopper, *Halticus tibialis* (Reuter) and the mustard aphid, *Liphaphis erysimi* (Davis). In this paper, we present the incidence of pests and soft rot disease in the cabbage fields with and without trap crops.

Materials and Methods

Experiments were conducted on two field plots (11.2 x 16.2 m) located 80 m apart from each other at the Inarajan agricultural farm. In each plot, 10 rows were transplanted with one month old cabbage seedlings cv. K K Cross at a spacing of 1.2 m between and 0.35 m within rows. Trap crops one row each of Chinese cabbage cv. Tempest, radish cv. Minowase Summer Cross 3, and Indian mustard, were planted on either side of the upwind plot. These trap crops were transplanted two weeks prior to the planting of cabbage seedlings.

Larvae of *H. undalis, C. pavonana* and *Spodoptera litura* (F.) and adults and nymphs of *H. tibialis* were counted from 10 plants selected at random in each row at weekly intervals for 10 weeks. There was no incidence of diamondback moth, *Plutella xylostella* (L.) and aphids during the period of this experiment.

Results and Discussion

H. undalis larvae were mostly found on radish and mustard in the cabbage plot with trap crops *(Figure 1)*. There was significantly more *H. undalis* on the cabbage plants in the plot without trap crops than the one with the trap crops *(Figure 2)*. The incidence of the disease, soft rot on cabbage in the nontrap cropped plot was 34.5% higher than in the trap cropped plot *(Figure 3)*. Al-Janabi *et al.* (1990) also found a positive relationship to *H. undalis* bore holes and associated secondary insect infestation to the incidence of soft rot in Kohlrabi in Iraq.

The incidence of *C. pavonana* was more on the trap crops than on cabbage in the trap cropped plot *(Figure 4)*. Heavy incidence of *C. pavonana* on mustard trap grown next to cabbage resulted in migration of the caterpillars from mustard to cabbage. Some caterpillars were also blown over by wind from the mustard to the cabbage plants *(Figure 5)*.

H. tibialis was found only on the trap crops. The heaviest infestation occurred on the radish. The trap crops used in this experiment did not have any attraction to *S. litura*. More *S. litura* larvae were found on cabbage than in the trap crops however there were more larvae in the cabbage plots without the trap crops than the one with the trap crops *(Table 1)*.

The trap crops, Chinese cabbage, radish and Indian mustard were effective in reducing the incidence of *H. undalis, C. pavonana* and *H. tibialis* in cabbage plantings. The reduction in infestation of *H. undalis* in cabbage also reduced the incidence of soft rot. Mustard row should be the outermost one in the trap crops or adequate spacing between cabbage and mustard should be given to avoid caterpillars being blown by wind on the cabbage plants.

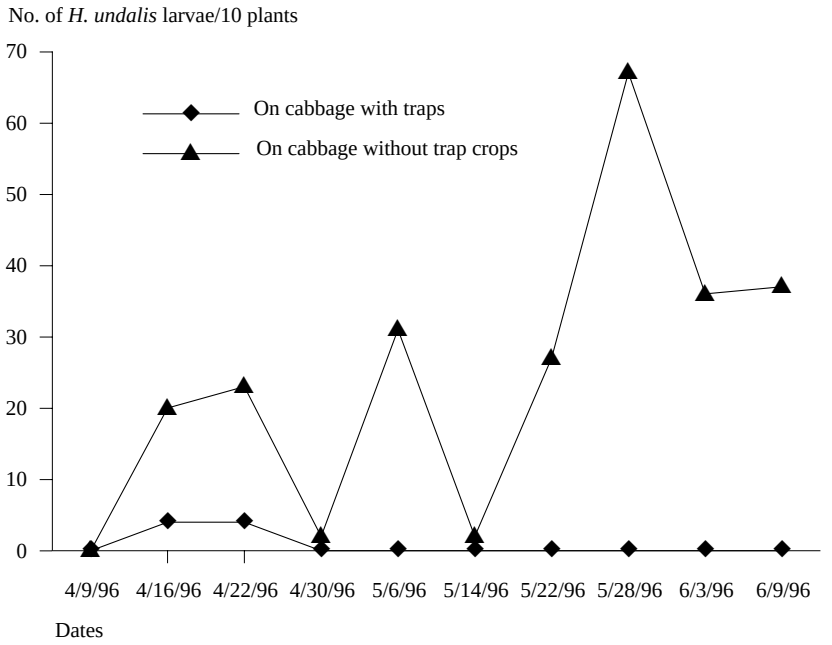


Figure 1. Hellula undalis larvae in the cabbage plots with and without trap crops

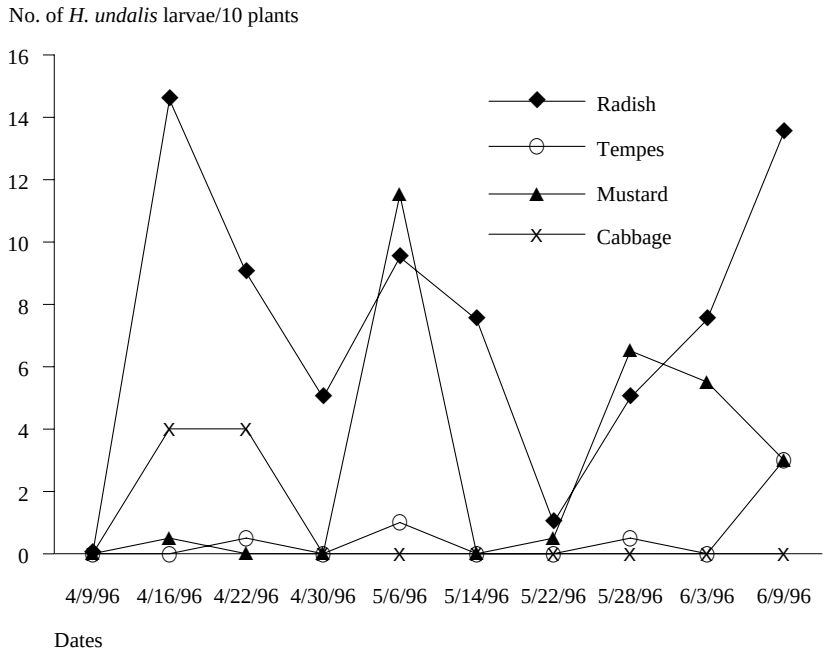


Figure 2. Hellula undalis larvae in the trap cropped field

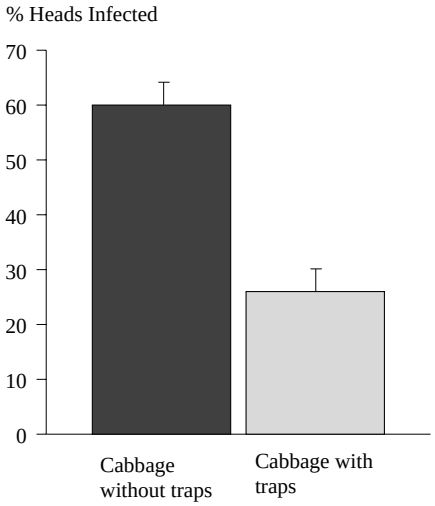


Figure 3. Cabbage heads infested with soft rot in the plots with and without trap crops

Table 1. Incidence of *Halticus tibialis* and *Spodoptera litura* in the cabbage field with and without trap crops

Insects/ treatments	*H. tibialis*		*S. litura*	
	Mean	S.E.	Mean	S.E.
Cabbage crop[1]	0	0	1.5	0.3
Cabbage crop[2]	0	0	3.9	0.5
Radish	26.1	0.4	0	0
Tempest	1.6	0.6	0.2	0.2
Mustard	2.7	0.5	0.2	0.2

[1]Cabbage with trap crops
[2]Cabbage without trap crops

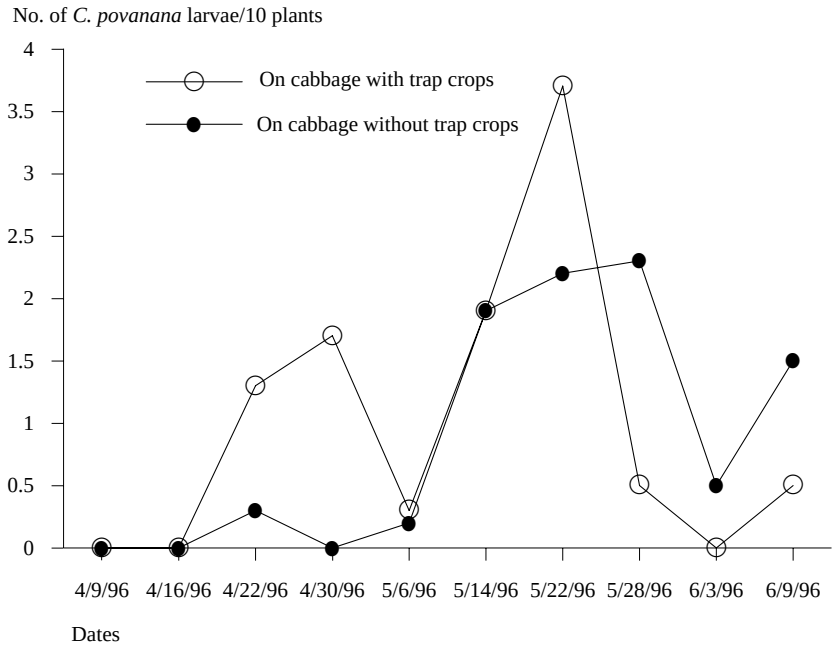


Figure 4. Crocidolomia pavonana larvae in the trap cropped field

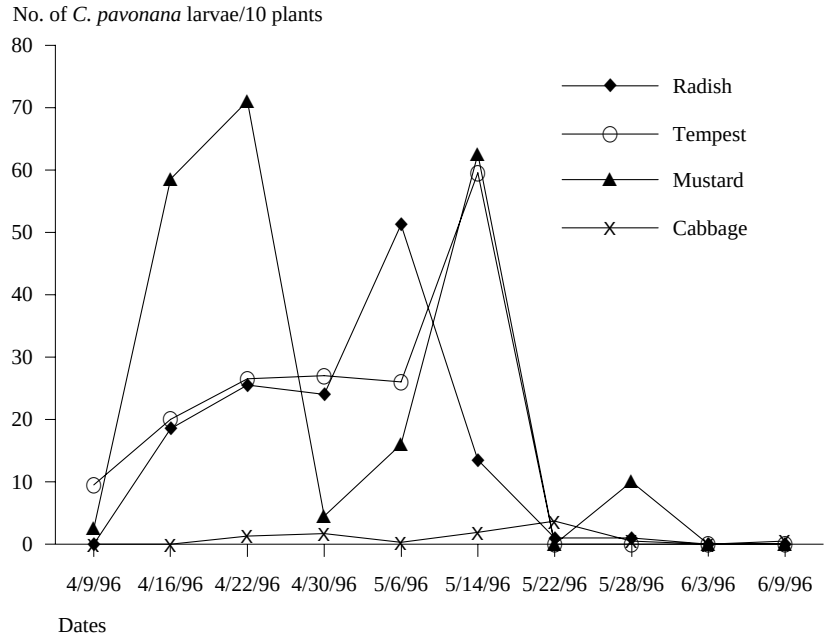


Figure 5. Crocidolomia pavonana larvae in cabbage plots with and without trap crops

Acknowledgement

Funding for research was provided by the project GUA000103 and in part by the USDA under CSREES Special Grant agreement No. 93–34135–8827 managed by Pacific Basin Administrative Group (PBAG).

References

Al-Janabi, G. D., Al-Azawi, A. F and Tamimi, T. (1990). Some important pests of kohlrabi and their possible role in dissemination of soft bacterium in Iraq. *Arab Journal of Plant Protection.* ***8:*** 25–29.

Hokkanen, H. M. T. (1989). Biological and agrotechnical control of the rape blossom beetle, *Meligethes aeneus* (Coleoptera, Nitidulidae). *Acta Entomologica Fennica* ***53:*** 25–29.

—— (1991). Trap cropping in pest management. *Annual Review of Entomology* ***36:*** 119–138.

Hokkanen, H., Granlund, H., Husberg, G. G., and Markkula, M. (1986). Trap crops used successfully to control *Meligethes aeneus* (Col., Nitidulidae), the rape blossom beetle. *Annales Entomologici Fennici* ***52:*** 115–120.

Muniappan, R. and Marutani, M. (1992). Pest management for head cabbage production on Guam. Proceedings of the Second International Workshop on Diamondback Moth and other Crucifer Pests, Tainan 1990, pp 541–549.

Silva-Krott, I. U., Singh, P., Lali, T. S., and Muniappan, R. (1995). Development of a trap cropping system for cabbage in Guam. *Pest Management in Horticultural Ecosystems* ***1:*** 27–35.

Srinivasan, K. and Krishna Moorthy, P. N. (1991). Indian mustard as a trap crop for management of major lepidopterous pests on cabbage. *Tropical Pest Management.* ***37:*** 26–32.

Waterhouse, D. F. (1993). The Major Arthropod Pests and Weeds of Agriculture in Southeast Asia. ACIAR, Canberra 141 pp.

Waterhouse, D. F. and Norris, K. R. (1987). Biological Control-Pacific Prospects. Inkata Press, Melbourne, 454 pp.

Waterhouse, D. F. and Norris, K. R. (1989). Biological Control-Pacific Prospects – Supplement 1, ACIAR, Canberra 123 pp.

Simulation model for forecasting population fluctuations of the diamondback moth in cabbage fields

Kazunobu Okadome

Kyoto Prefectural Agricultural Research Institute, 9 Wakunari Amarube-cho Kameoka-shi Kyoto 621, Japan

Abstract

In order to implement an integrated pest management (IPM) program for the diamondback moth (DBM), I attempted to develop a simulation model to forecast DBM population fluctuations in cabbage fields. The egg density was estimated by a regression equation between the number of moths caught by a pheromone trap and the egg density in cabbage fields. The development of immature stages was started from each date of oviposition. Developmental velocity was assessed by a thermal constant using the daily mean temperature. Survival of immature stages was assumed to be affected by rainfall and insecticide spraying. The effects of the mortality factors were numerically evaluated using previously reported data. The number of individuals in each stage was calculated on a daily basis with a matrix model using a personal computer. From the results of a validity test, the model predicted well the actual population fluctuations in a cabbage field treated with pesticides in the cultivation period from spring to early summer, but it was not successful in predicting these fluctuations in autumn. Although the model needs to be improved in the future, it will be a useful tool for implementation of an IPM system for the DBM.

Key words: simulation model, *Plutella xylostella*, IPM, cruciferous vegetable

Introduction

The diamondback moth (DBM), *Plutella xylostella* (L.), is one of the important pests of cruciferous vegetables that are grown extensively in spring, early summer and autumn in Kyoto, Japan. These vegetables include, cabbage, chinese cabbage, komatuna, mizuna and mibuna, the latter two being the traditional vegetables in Kyoto. In order to control the DBM, various insecticides have been frequently sprayed or dusted or incorporated into the soil. Under the conditions of repeated use of insecticides, the DBM has developed resistance against most of them including the microbial insecticide *Bt*. Another problem is that the control cost increases the production price of the vegetables. In addition, we fear that the heavy use of pesticides may affect the environment.

In order to improve the current control method, an integrated pest management (IPM) system is required for the DBM. The definition of IPM includes three key concepts: integration of plural control measures, a determination of economic injury level, and a population management system (FAO, 1967). A systems model is needed to forecast fluctuations in the populations of insect pests to practice the latter two concepts. However such a systems model has not been developed for the DBM. In the present study, I attempted to develop a simulation model to describe the processes of population fluctuation of the DBM.

Model Structure

The model is based on a matrix model like that of Leslie (1945). The biological framework of the model is shown in *Fig. 1*.

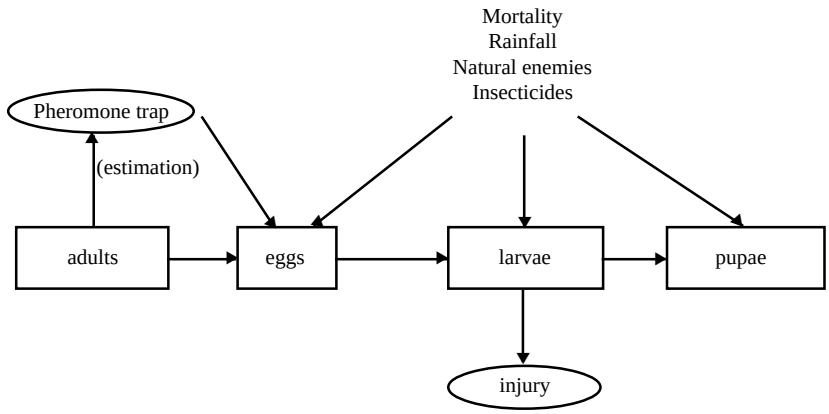


Figure 1. Conceptual flow chart of the simulation model of DBM in a field

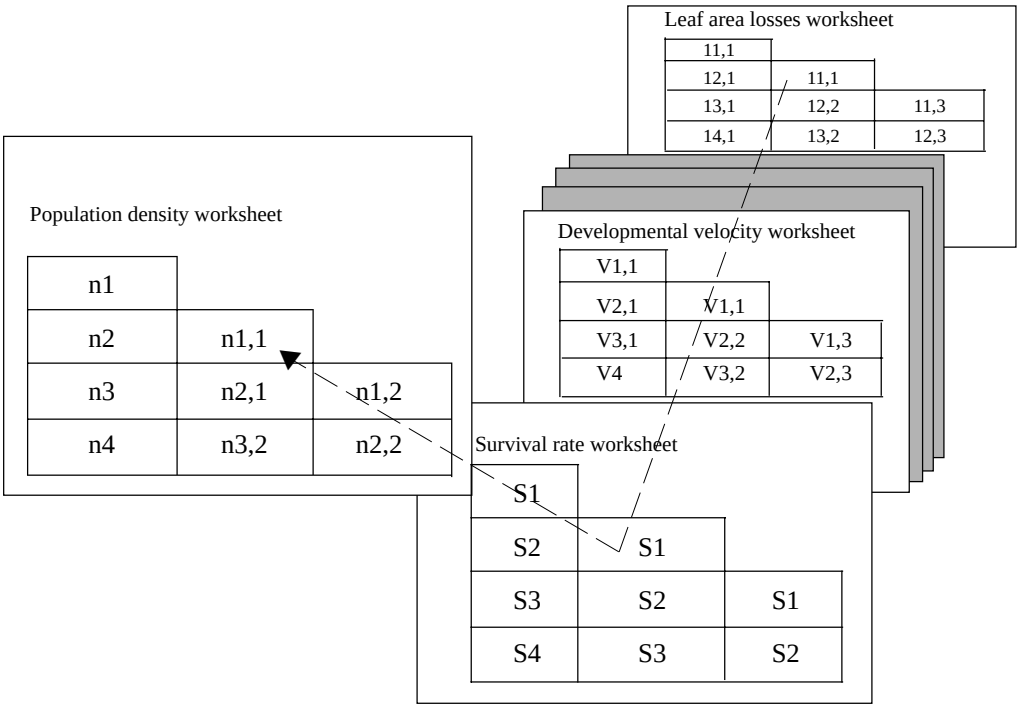


Fig. 2. Basic structure of the DBM model. n, S, V and l refer to density of DBM, leaf area losses of cabbage, survival rate and developmental velocity of DBM, respectively. The arrow shows how the data in different worksheets are linked.

The biological data and parameters of mathematical equations involved in the model were obtained from experiments that were conducted in the laboratory or in the cabbage fields by the author (unpublished data), and also from the literature.

The number of individuals in each stage was calculated on a daily basis by a matrix model using a personal computer. Macroinstructions of a spreadsheet program (Lotus 1-2-3® for Windows®) were used. The model structure is shown in *Fig. 2.* Four tables in four worksheets with the same framework were prepared to describe the DBM larvae: (1) population density, (2) developmental velocity, (3) survival rate, and (4) leaf area losses of cabbage.

The cells are arranged in the same format on each of the worksheets, and each cell location on the four worksheets corresponds to the data on the *t*th day and on the *k*th day of age.

Description of the population density

The second column from left on the sheet for population density *(Fig. 2)* is used to input the egg density laid on the *t*th day. The population density from the eggs on the *t*th day changes along the diagonal direction as shown in *Fig. 3.* The total incidence on the *t*th day is obtained by the summation of the densities in all cells in the horizontal direction as shown in *Fig. 4.*

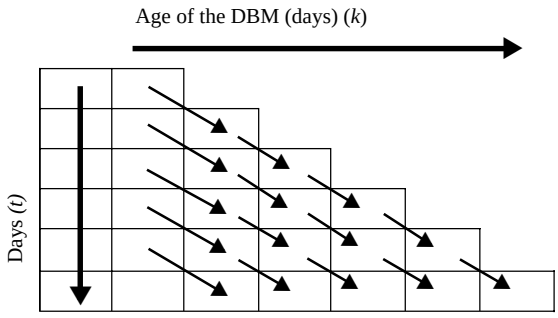


Fig. 3. Direction of the changes in the DBM population started from the eggs laid on each day.

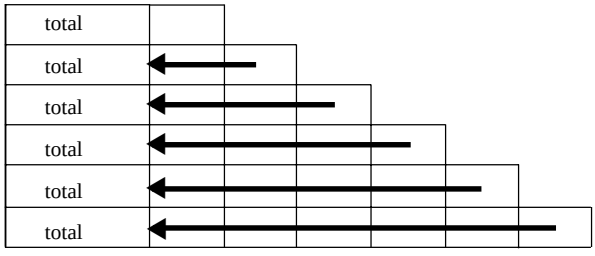


Fig. 4. Method for calculating the total density of the DBM.

	0 day age →	1 day age →	2 days age →	3 days age →	4 days age → ..... k days age	
1st day N_1	$n_{1,0} = e_1$					
2nd day N_2	$n_{2,0} = e_2$	$n_{1,1} = n_{1,0} \times S_{1,1}$				
3rd day N_3	$n_{3,0} = e_3$	$n_{2,1} = n_{2,0} \times S_{2,1}$	$n_{1,2} = n_{1,1} \times S_{1,2}$			
4th day N_4	$n_{4,0} = e_4$	$n_{3,1} = n_{3,0} \times S_{3,1}$	$n_{2,2} = n_{2,1} \times S_{2,2}$	$n_{1,3} = n_{1,2} \times S_{1,3}$		
5th day N_5	$n_{5,0} = e_5$	$n_{4,1} = n_{4,0} \times S_{4,1}$	$n_{3,2} = n_{3,1} \times S_{3,2}$	$n_{2,3} = n_{2,2} \times S_{2,3}$	$n_{1,4} = n_{1,3} \times S_{1,4}$	
⋮	⋮	⋮				
t^{th} day N_t	$n_{t,0} = e_t$	$n_{t-1,1} = n_{t-1,0} \times S_{t-1,1}$	$n_{t-2,2} = n_{t-2,1} \times S_{t-2,2}$	$n_{t-3,3} = n_{t-3,2} \times S_{t-3,2}$	$n_{t-4,4} = n_{t-4,3} \times S_{t-4,4}$ •••••	$n_{1,k} = n_{1,k-1} \times S_{1,k}$

Fig. 5. Expressions used to calculate the population fluctuations.
$N_t = n_{t,o} + n_{t-1,1} + n_{t-2,2} + \cdots\cdots + n_{1,k}$, e_t: the number of eggs laid on tth day, $n_{1,k}$: density at the age (k) in days starting from eggs laid on the first day, $S_{1,k}$: survival rate on the kth day of age of the population was oviposited on the first day.

Changes in population density from the *t*th day to the (*t*+1)th day are described as follows:

$$\begin{bmatrix} S_{t,o} & O & O & \cdots & O & O \\ O & S_{t,1} & O & \cdots & O & O \\ O & O & S_{t,2} & \cdots & O & O \\ \vdots & \vdots & \vdots & & \vdots & \vdots \\ O & O & O & & S_{t,k} & O \end{bmatrix} \begin{bmatrix} e_t \\ n_{t,1} \\ n_{t,2} \\ \vdots \\ n_{t,k} \end{bmatrix} = \begin{bmatrix} n_{t+1,1} \\ n_{t+1,1} \\ n_{t+1,3} \\ \vdots \\ n_{t+1,k+1} \end{bmatrix}$$

where (e_t)is the egg density laid on the *t*th day, and $n_{t,k}$ and $S_{t,k}$ are the population density and survival rate on the *t*th day at the *k*th age, respectively.

The population density ($n_{t,k}$) is calculated by using the data given in the worksheet in *Fig. 2*. The calculation procedures are shown in *Fig. 5*.

Estimation of egg density

Because of the difficulty in directly observing egg density of the DBM in cabbage fields, an attempt was made to estimate egg density from the number of moths caught by a pheromone trap (e.g., Nakasuji and Kiritani, 1978 and Kondo and Tanaka, 1995). However, a reliable relationship between the egg density and the trap catches was not obtained for the DBM.

The relationship between the index *(IT)* of trap catches (daily number of moths caught *(AT)* multiplied by the survival rate *(S)* of immature stages, *(IT=AT•S)* and the mean daily density *(NL)* of larvae observed in a cabbage field is shown in *Fig. 6.* Since *NL* is given as the product of the egg density *(E)* and survival rate *(S) (NL=E•S)*, the regression equation *(E•S=a•AT•S)* should give the relationship between the trap catches and egg density. In this case, the equation should pass through the origin. Although the intercept on the axis

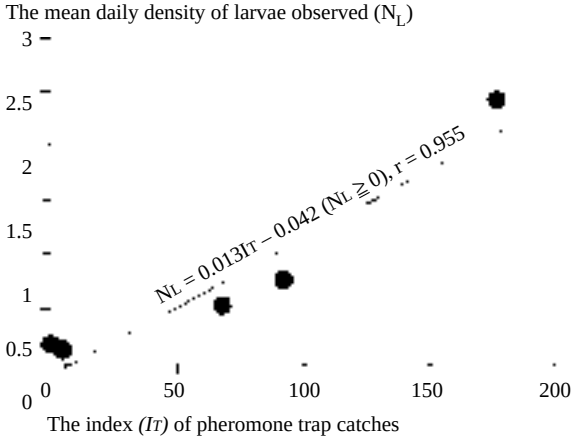


Fig. 6. The relationship between Index of trap catches (IT) and Mean daily density of larvae (NL) observed in a cabbage field.

Data was obtain by the number (AT) of moths caught multiplied by the survival rate (S) of larvae (IT=AT•S). The survival rates were simulated by the model under the same conditions in the observed cabbage field. The larval density (NL) was the observed value but the density was assumed to be the egg density (E) multiplied by the survival rate (S) (E•S), where S was the same as mentioned above. Therefore, we can estimate the egg density (E) by using the number of moths caught (AT) by the pheromone trap into the dependent variable (IT) of the regression equation in the figure.

was not zero, the regression equation, e_t=0.013ATt-0.041 ($e_t \geqq 0$), r=0.955 (p<0.05, t-test) was tentatively used to estimate the number of eggs (e_t) laid on the *t*th day, where ATt is the daily number of moths caught by the pheromone trap. This equation should be replaced by a direct regression between the trap catches and egg density in the future.

Survival rate

The survival of immature stages of the DBM is affected by rainfall, natural enemies and insecticide spraying. The effects of these mortality factors were numerically evaluated using previously reported data. The rainfall mortality data were cited from Wakisaka *et al*. (1991), but the data were modified as follows:

S = 0.005P (egg),
S = 1–0.004P (the 1st instar of larvae),
S = 1–0.004P (2nd instar),
S = 1–0.005P (3rd instar),
S = 1–0.002P (4th instar),
S = 1–0.001P (pupae)

where S is the percent of the larvae that survive the rainfall, and P is the precipitation (mm) on each day. The data for stage-specific mortality from insecticide spraying were based on an unpublished study by Yoshikimi Hayashida (Kyoto Prefectural Agricultural Research Institute). The data for mortality from rainfall and insecticide spraying were calculated by a spreadsheet function as follows:

@IF (V_{tk} <V_u*#and#* V_{tk} > = V_L,M_{st},*O)*

This function returns a mortality value (M_{st}) of an individual at a particular stage on the *t*th day when the cumulative developmental velocity (V_{tk}) was kept between an upper limit (V_u) and a lower limit (V_L) for that stage, and returns a value of 0 when V_{tk} was outside this range.

Since the mortality from natural enemies was not been evaluated numerically, the effects of natural enemies was not simulated in the present study.

In the model, the different survival rates for each stage of the DBM were given on the different sheets *(Fig. 7).*

Developmental velocity

The development of immature stages is started from each date of oviposition. The developmental velocity was assessed by a thermal constant using the daily mean temperature. The following regression equations between developmental velocity (V) and temperature (T) were used:

V = 0.026T-0.271, r = 0.917 (from oviposition to hatching)
V = 0.007T-0.076, r = 0.927 (from oviposition to pupation)
V = 0.005T-0.054, r = 0.936 (from oviposition to adult emergence)

These equations are incorporated in the spreadsheet for developmental velocity to give the cumulative developmental velocity for each day and for each age *(Fig. 8).*

Therefore, completion of a developmental stage can be calculated using the spreadsheet function as follows:

@if(V>=1,0,n)

which outputs the calculated population density *(n)* if V<1 and 0 when the cumulative developmental velocity *(V)* reaches 1 or more. A value of 0 means the completion of development in each stage.

In each cell on the sheet to estimate development *(Fig. 8),* the developmental stage is calculated by this method.

Leaf area losses of cabbage by larval feeding

The relationship between the mean cumulative leaf area loss *(L)* of cabbage by feeding of a larva and the larva's cumulative developmental velocity *(V)* is shown in *Fig. 9.*

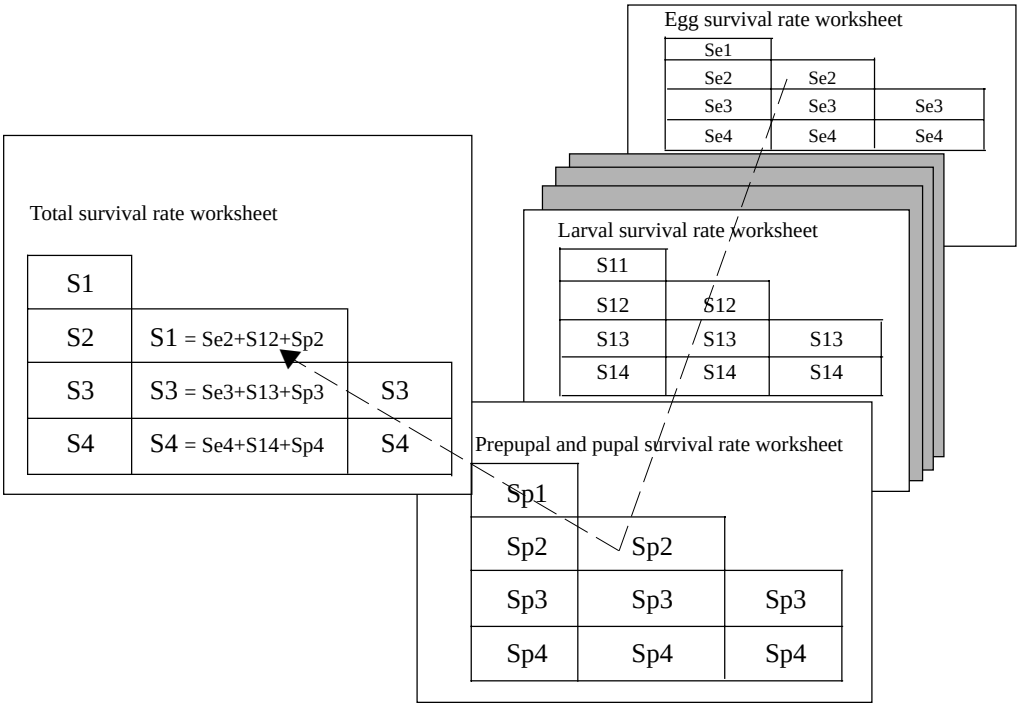


Fig. 7. The basic structure for calculating survival rate of DBM. Each cell on each sheet outputs survival rate when the development of DBM reaches the stage shown on each sheet.

	0 day of age →	1 day of age →	2 days of age →	3 days of age →	4 days of age →	.....	k days of age
1st day	0						
2nd day	0	$V_{1,1} = aT_1 + b$					
3rd day	0	$V_{2,1} = aT_2 + b$	$V_{1,2} = aT_2 + b + V_{1,1}$				
4th day	0	$V_{3,1} = aT_3 + b$	$V_{2,2} = aT_3 + b + V_{2,1}$	$V_{1,3} = aT_3 + b + V_{1,2}$			
5th day	0	$V_{4,1} = aT_4 + b$	$V_{3,2} = aT_4 + b + V_{3,1}$	$V_{2,3} = aT_4 + b + V_{2,2}$	$V_{1,4} = aT_4 + b + V_{1,3}$		
⋮		⋮					
tth day	0	$V_{t-1,1} = aT_{t-1} + b$	$V_{t-2,2} = aT_{t-2} + b + V_{t-2,1}$	$V_{t-3,3} = aT_{t-3} + b + V_{t-3,2}$	$V_{t-4,4} = aT_{t-4} + b + V_{t-4,3}$	•••	$V_{1,k} = aT_{t-1} + b + V_{1,k-1}$

Fig. 8. Expressions used in the worksheet for cumulative developmental velocity. Cumulative developmental velocity (V) *at kth age that was oviposited on the first day is calculated from the accumulated daily developmental velocity (See text).*

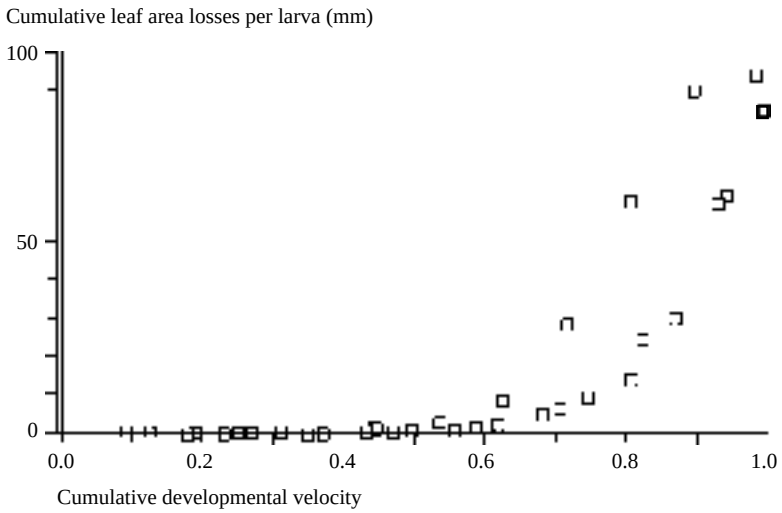


Fig. 9. Relationship between development of the DBM and cumulative leaf area losses of cabbage.

Leaf area loss was obtained by the conditional polynomial regression equation as follows:

$L = 317.39V^3 - 257.50V^2 + 53.18V - 1.45$ $(V \geq 0.486)$

$L = 0$ $(V < 0.486)$

$r_2 = 0.888$

The mean leaf area loss (l_t) on the *t*th day is calculated by:

$l_t = n_t[317.39(V_t^3 - V_{t-1}^3) - 257.50(V_t^2 - V_{t-1}^2) + 53.18(V_t - V_{t-1})]$ $(V_t \geqq 0.486)$

$l_t = 0$ $(V_t < 0.486)$

where V_t is the cumulative developmental velocity of DBM on the *t*th day and n_t is the population density on the *t*th day. To calculate leaf area losses by the feeding of larvae , these expressions were placed in the cells on the worksheet for leaf area losses *(Fig. 2)*.

Validity test of the model

The model proposed in the present study can predict the population density of the DBM in each stage and the losses of cabbage leaves in cabbage fields, if pheromone trap data and precipitation and mean temperature data (from meteorological records) are provided.

The predicted values of the population densities of each stage as well as the total density and leaf area losses in the cabbage field where insecticide was not sprayed, and a comparison between the estimated values and the observed values of the density are shown in *Fig. 10*. In addition, the predicted and observed values in the cabbage field where insecticides were sprayed are compared in *Fig. 11*. The census was conducted in the cabbage fields in spring to early summer in 1994. The mean temperature and precipitation used in *Fig. 10* were obtained from the records of the Kyoto Prefectural Agricultural Research Institute, and those used in *Fig. 11* were obtained from the Kyoto Meteorological Observatory.

The predicted values of DBM density were considerably higher than those in the field without insecticide spraying. On the other hand, the values predicted by the model coincided well with the observed density in the cabbage field with insecticide spraying. Because I did not conduct the censuses for the leaf area losses in the field, the validity of the model was not tested for the leaf area losses. In autumn in 1995, the model failed to predict the actual densities (data not shown).

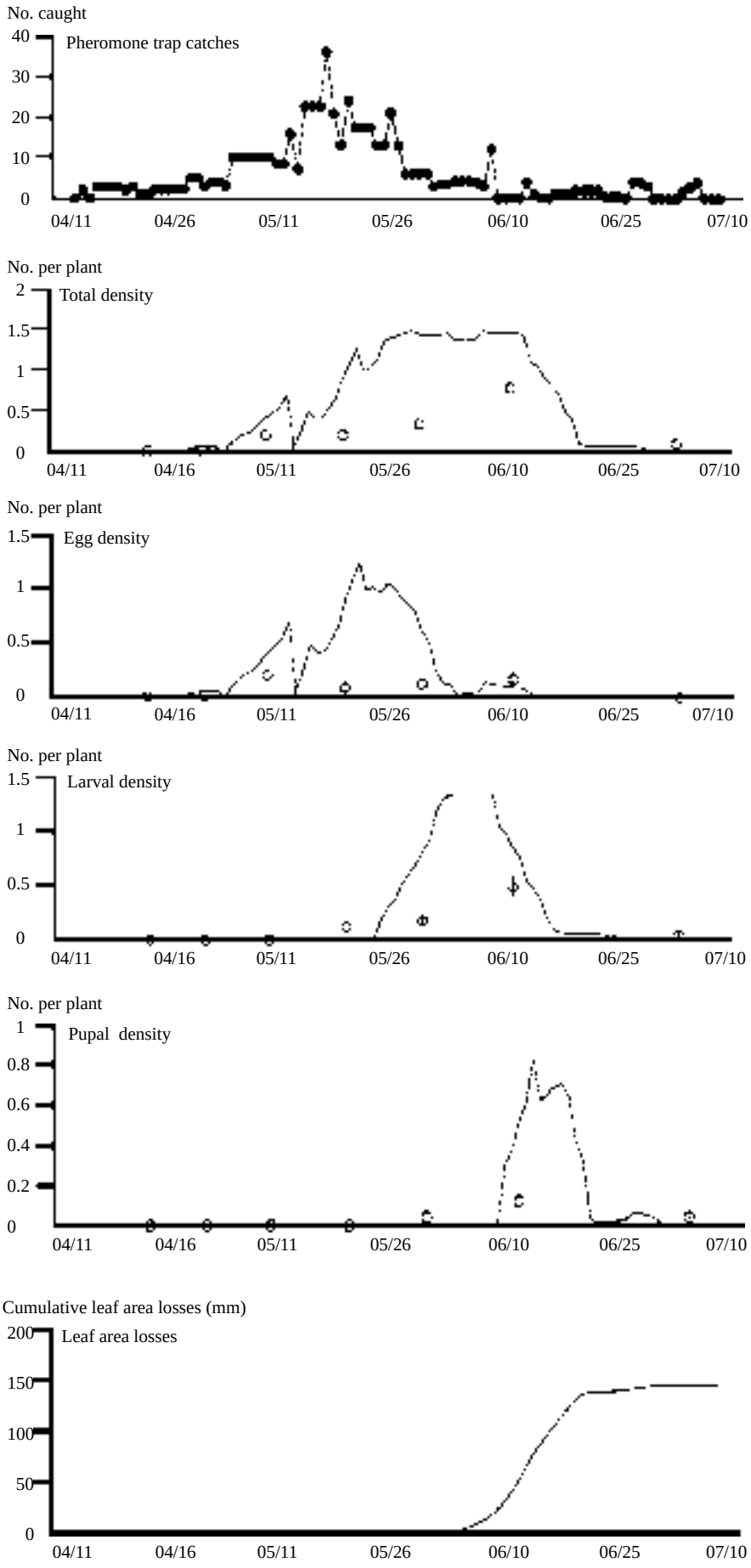
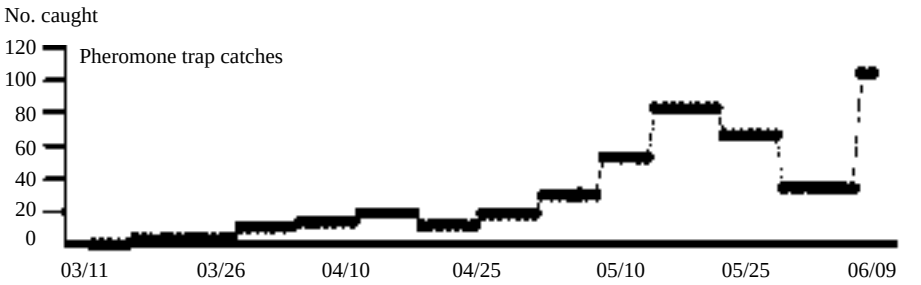
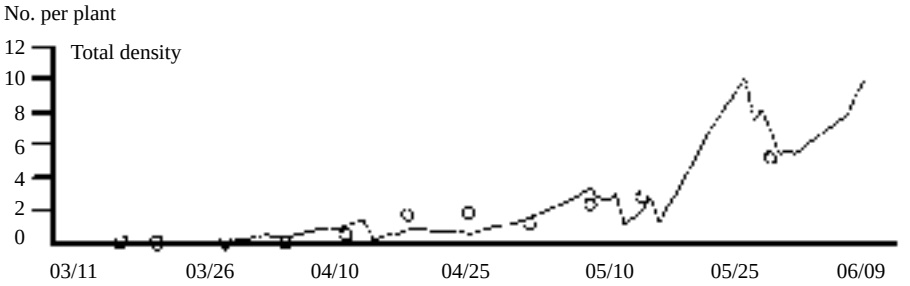
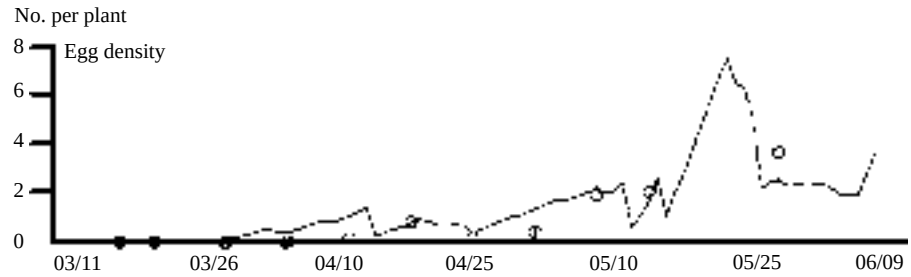
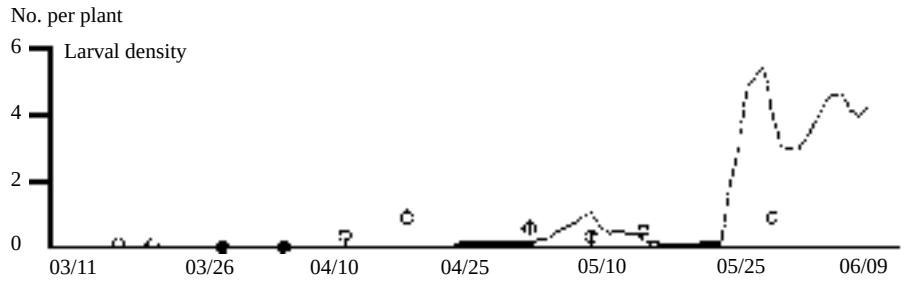


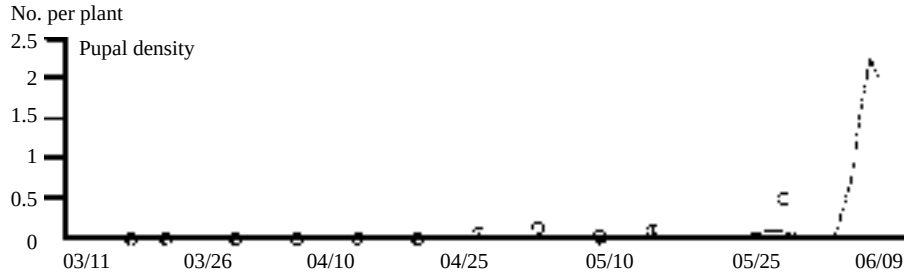
Fig. 10. Predicted and observed values of the population densities of the DBM, and leaf area losses of cabbage in a cabbage field without insecticide spraying (1994).

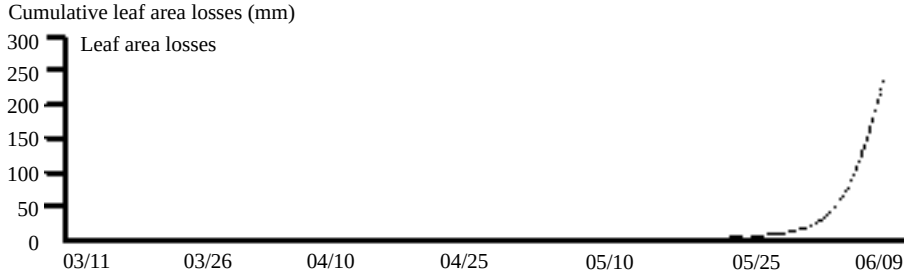

No. caught
Pheromone trap catches
120
100
80
60
40
20
0
03/11
03/26
04/10
04/25
05/10
05/25
06/09


No. per plant
Total density
12
10
8
6
4
2
0
03/11
03/26
04/10
04/25
05/10
05/25
06/09


No. per plant
Egg density
8
6
4
2
0
03/11
03/26
04/10
04/25
05/10
05/25
06/09


No. per plant
Larval density
6
4
2
0
03/11
03/26
04/10
04/25
05/10
05/25
06/09


No. per plant
Pupal density
2.5
2
1.5
1
0.5
0
03/11
03/26
04/10
04/25
05/10
05/25
06/09


Cumulative leaf area losses (mm)
Leaf area losses
300
250
200
150
100
50
0
03/11
03/26
04/10
04/25
05/10
05/25
06/09

cont.

Figure 11. Cont.

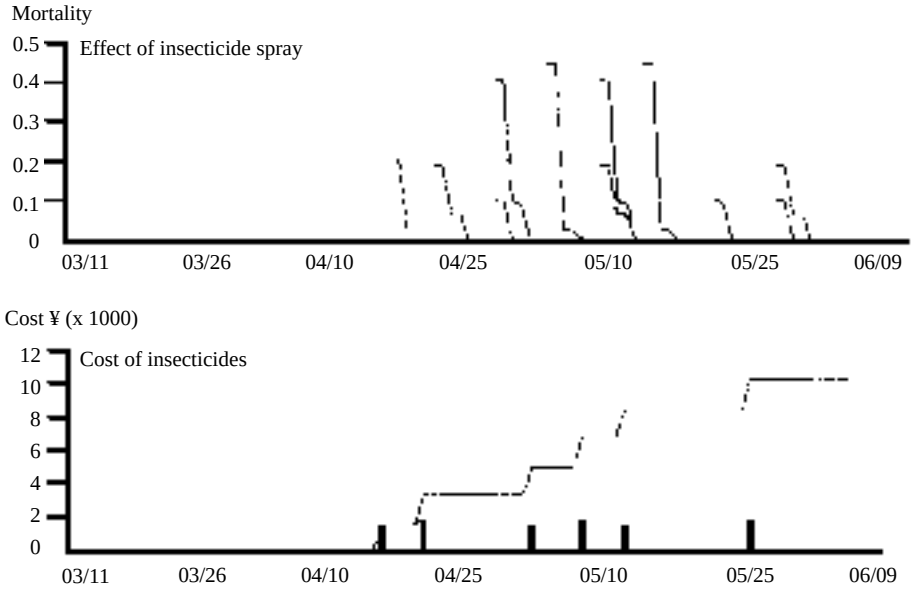


Fig. 11. Predicted and observed values of the population densities of the DBM, leaf area losses of cabbage, effect of insecticide spraying and cost of insecticides (1994).

Discussion

The objective of the present study was to develop an easier forecasting method for population fluctuations of the DBM and to assess the crop losses due to the DBM using a personal computer. The model was programmed using spreadsheet software. Functions for making up graphs, auto calculation, data links, etc. were prepared in the spreadsheet software. Therefore, the data are easily entered and the results are easily obtained.

Due to the lack of information on the biological processes that determine DBM abundance, an exact prediction of the population density was not made by the present model. Especially, data on mortality from natural enemies and on the residual effects of insecticide spraying are necessary to improve the model. Regarding injury to the cabbage, the level of injury that a cabbage plant can tolerate should be determined for the practical use of the present model.

Acknowledgments

I wish to thank Prof. Fusao Nakasuji (Okayama University) and Dr. Sugio Yanase (Kyoto Prefectural Agricultural Research Institute) for their valuable suggestions and critical reading of the manuscript. I also thank Dr. James Raymond (Eigo Kenkyushitsu) for checking grammatical constitution of the manuscript. This study was partially supported by Grants from the Division of Plant Protection, Ministry of Agriculture, Forestry and Fisheries, Japan.

References

FAO (1966). Report of the FAO / UNEP panel of experts on integrated pest control 1965, Rome. Part 1, 91pp; Part 2, 186pp; Part 3, 129pp.

Kondo, A. and Tanaka, F. (1995). An estimation of the control threshold of the rice stem borer, *Chilo suppressalis* (Walker) (Lepidoptera: Pyralidae) based on pheromone trap catches. *Appl. Entomol. Zool.* ***30(1):*** 103–110

Leslie, P. H. (1945). On the use of matrices in certain population mathematics. *Biometrika* ***33:*** 183–212

Nakasuji, F. and K. Kiritani (1978). Estimating the control threshold density of the tobacco cutworm *Spodoptera litura* (Lepidoptera: Noctuidae) on a corm crop, taro, by means of pheromone traps. *Prot. Ecol. 1:* 23–32

Wakisaka, S., R. Tsukuda and F. Nakasuji (1991). Life table of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) and effects of rainfall, temperature and host plant on survival and reproduction. *Jpn. J. Appl. Entomol. Zool.* ***30(1):*** 103–110 (In Japanese, English summary)

Improving IPM decisions for the management of diamondback moth on cabbages using sequential sampling plan

Chua Tock Hing and A. Sivapragasam

Department of Zoology, Universiti Malaya, 50603 Kuala Lumpur, Malaysia
Strategic, Environment and Natural Resources Centre, MARDI,
G.P.O, Box 12301, 50774 Kuala Lumpur, Malaysia.

Abstract

A sequential sampling plan, based on the negative binomial distribution of the insect, was developed for use in the IPM of diamondback moth (DBM) (*Plutella xylostella* L.) in Malaysia. The value of the common k (=1.84) was calculated from weekly sampling of 60 plants selected randomly from cabbages grown without the use of insecticides. Two sets of sequential sampling lines, representing two action thresholds (7 and 14 DBM larvae per plant, respectively) for two different stages of the crop age (< 1 month and > 1 month respectively) were formulated: $d_0 = 5.26\ n - 15.14$, $d_1 = 5.26\ n + 15.14$ and $d_0 = 10.61n - 6.68$, $d_1 = 10.61n + 6.68$, respectively, where 0 = no treatment required and 1 = insecticide application required; n= number of samples taken, a and b (both are probabilities of error) = 0.1. An appraisal of the plan using historical data on three treatments (IPM, prophylatic insecticide application and ecological control) indicated that if treatment is required, it could be detected at the 5th sample, thus making a saving up to 75% of the sampling cost and 60% of insecticide and labour costs.

Key words: sequential sampling, diamond back moth, cabbage

Introduction

Sequential sampling was first developed by Wald (1945), although not for biological purposes. It has the main characteristic of a changing sampling size for estimating the population density, the sampling size being determined as sampling progresses. With reference to its use in insect pest management, sequential sampling is a procedure whereby samples are taken in sequence with pest management decisions being made on the information obtained from each observation. Thus, sequential sampling is designed simply to answer the question whether the estimated pest density is lower or higher than a preset critical level which is normally the threshold level for taking control measures against the pest. Sampling will continue until the data indicate that one alternative (eg not to apply insecticide) is more likely true than the other at a specified acceptable level of probability. In this respect it is rather efficient as very few observations are needed when the insect populations are very low or very high, and extensive sampling is required only at intermediate density levels. Thus when infestation is low or high, the cost of sampling is reduced as fewer samples will be taken.

Sequential sampling plans have been developed and used in the Integrated Pest Management (IPM) programmes of many insect pests. The notable ones are for spruce budworm (Morris, 1954), red-pine sawfly (Connola *et al.*, 1959), cabbage looper (Harcourt, 1966a), cabbage worm (Harcourt, 1966b), green cloverworm (Hammond and Pedigo, 1976), cereal aphids (Ba-angood and Stewart, 1980), rice planthoppers (Shepard *et al.*, 1986), Malayan black bug (Ferrer and Shepard, 1987) and gypsy moth (Fleischer *et al.*, 1991).

We present here the results of a sequential sampling plan for diamondback moth, *Plutella xylostella* L. (DBM) which we believe could be used beneficially in the IPM of DBM. It will save cost of managing the pest by (i) reducing the number of samples needed to make an IPM decision whether or not to implement control measures (such as application of insecticides), thus speeding up the decision making process, and (ii) reducing the frequency of taking control measures.

Materials and Methods

Development of sequential sampling plan

Prerequisites for developing a sequential sampling plan for the diamond back moth include knowledge of type of spatial distribution (*e.g.* regular, random or aggregated), economic thresholds or pest density treatment levels, and an acceptable probability of error in the ultimate decision (Pieters, 1978).

In sequential sampling, two types of error are involved viz. the probability (α) of recommending an unnecessary treatment and the probability (β) of failing to recommend one when needed. In statistical terms these are also known as type I and II errors, respectively. In layman's language, they may be interpreted as: α = risk of concluding that the infestation is high when it is in fact low, and β = risk of concluding that the infestation is low when it is high. Usually, α and β are set at 0.1 (*e.g.* Waters, 1955), although Shepard *et al.* (1986) and Ferrar and Shepard (1987) set it at 0.2.

Since the DBM larvae exhibited negative binomial distribution (Chua and Lim, 1979), the equations of the decision lines (d_0, d_1) (Oakland, 1950) are:

$d_1 = bn + c_1$ (upper line) and
$d_0 = bn + c_0$ (lower line)

where d_i = the cumulative number of insects,
subscripts 1 = upper line, 0 = lower line,
n = number of sampling units (whole plants) examined
b = slope of the lines
c_i = intercepts

The values of b, c_0 and c_1 are calculated using:

$$b = k \log (q_1/q_0) / \log (p_1 q_0 / p_0 q_1)$$

$$c_0 = \log [\beta / (1-\alpha)] / \log (p_1 q_0 / p_0 q_1)$$

$$c_1 = \log [(1-\beta)/ \alpha] / \log (p_1 q_0 / p_0 q_1)$$

where $p_0 = \overline{X}_0 / k$, $q_0 = 1 + p_0$; $p_1 = \overline{X}_1 / k$, and $q_1 = 1 + p_1$

$\overline{X}$= mean number of insects or the economic threshold of the insects and

k = estimated dispersion parameter = $\overline{X}_2 / (s_2 - \overline{X}$ (Bliss and Fisher, 1953).

The decision lines *(Figure 1)* constitute the criteria for rating the insect infestation. The plants are sampled and the number of insects are plotted as a series of points until the points cross (*i.e.* lie outside) either of the parallel lines. If the top line (d_1) is crossed, the infestation warrants control measures, and if the lower line (d_0) is crossed, no pest management action is necessary.

Associated with the sequential plan are the operating characteristic curve and the average sample number curve. However, these are not essential to the working of a sequential sampling programme even though they help in visualising its performance. The operating characteristic curve shows how the probability of not recommending treatment varies with the insect density, whereas the average sample number curve indicates the number of samples required for the sequential sampling plan at different infestation levels.

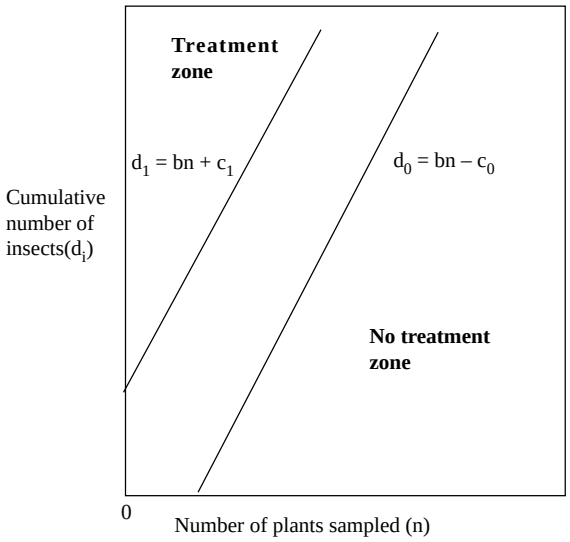


Figure 1. Sequential sampling graph for larvae of diamond back moth on cabbages for two crop ages.

Source of biological data

The IPM programme recommended by the Malaysian Agricultural Research and Development Institute (MARDI) consists of three packages (Loke *et al.*, 1996): Package I: economic threshold is 4 larvae per plant or 7 larvae with parasitism rate of at least 40% as a subparameter; the parasitism rate is based on dissecting 20 instar II-IV larvae; Package II: same as Package I except it takes into consideration the crop phenology. Since Package II *(Table 1)* was found to be the most superior in terms of yield and net profit, it was used as the basis of this sequential sampling plan. However to simply matters especially from the viewpoint of the farmers' use, the rate of parasitism (mainly by *Cotesia plutellae* Kurdjumov) was not included in the development of the plan.

The data on the spatial distribution of larvae was collected from three cabbage plots in MARDI Research Station (Jalan Kebun, Kelang) each measuring 15 m x 1.3 m. Each plot contained 50 cabbage plants grown with standard agronomic practices, and without the use of insecticides. Each week, 20 plants per plot were randomly sampled and the number of larvae and pupae of DBM per plant was recorded.

The caculation of k, and other parameters for the development of sequential sampling plan was done using Excel version 5 on the MacIntosh computer. The values for plotting the operating characteristic curve and the average sample number curve were determined using the methods discussed by Onsager (1976).

For the purpose of evaluating the effectiveness of the sequential sampling plan, data collected from an IPM trial conducted at the MARDI Research Station (Jalan Kebun, Kelang) in December 1989 - February 1990 was used. The trial consisted of three treatments, each replicated three times in a randomized complete block design. The treatments were IPM (package II), prophylactic practices and ecological plot (without the use of insecticides). Each cabbage plot measured 15 m X 1.3 m, and contained 500 cabbage plants. Standard agronomic practices were used. Each week, 20 plants per plot were sampled randomly and the number of larvae and pupae of DBM per plant was recorded. IPM decisions were based on the mean values of three plots.

Results and Discussion

The common value for k, the dispersion index of the negative binomial distribution, was found to be 1.84. With this, and setting α and β both equal to 0.1, the decision equations were determined to be as follows:

For crop age < 1 month, and economic threshold of 7 DBM larvae per plant:

$d_0 = 5.26\ n - 15.14$ and $d_1 = 5.26\ n + 15.14$ *(Figure 2).*

For crop age > 1 month, and economic threshold of 14 DBM larvae per plant

$d_0 = 10.61n - 6.68$ and $d_1 = 10.61n + 6.68$

For field use by the farmers, the sequential sampling plan could be converted into a table form *(Table 2)*, with which the cumulative DBM larvae

Table 1. Summary of IPM Package II for the management of diamondback moth (DBM) as recommended by MARDI (Malaysian Agricultural Research and Development Institute).

Crop age	DBM larvae/plant	Parasitism	Treatment
< 1 month	<4	not considered	not required
	4–7	>40%	not required
	4–7	<40%	apply *Bacillus thuringiensis*
	>7	not considered	apply pyrethroid
> 1 month	<8	not considered	not required
	8–14	>40%	not required
	8–14	<40%	apply *Bacillus thuringiensis*
	>14	not considered	apply pyrethroid

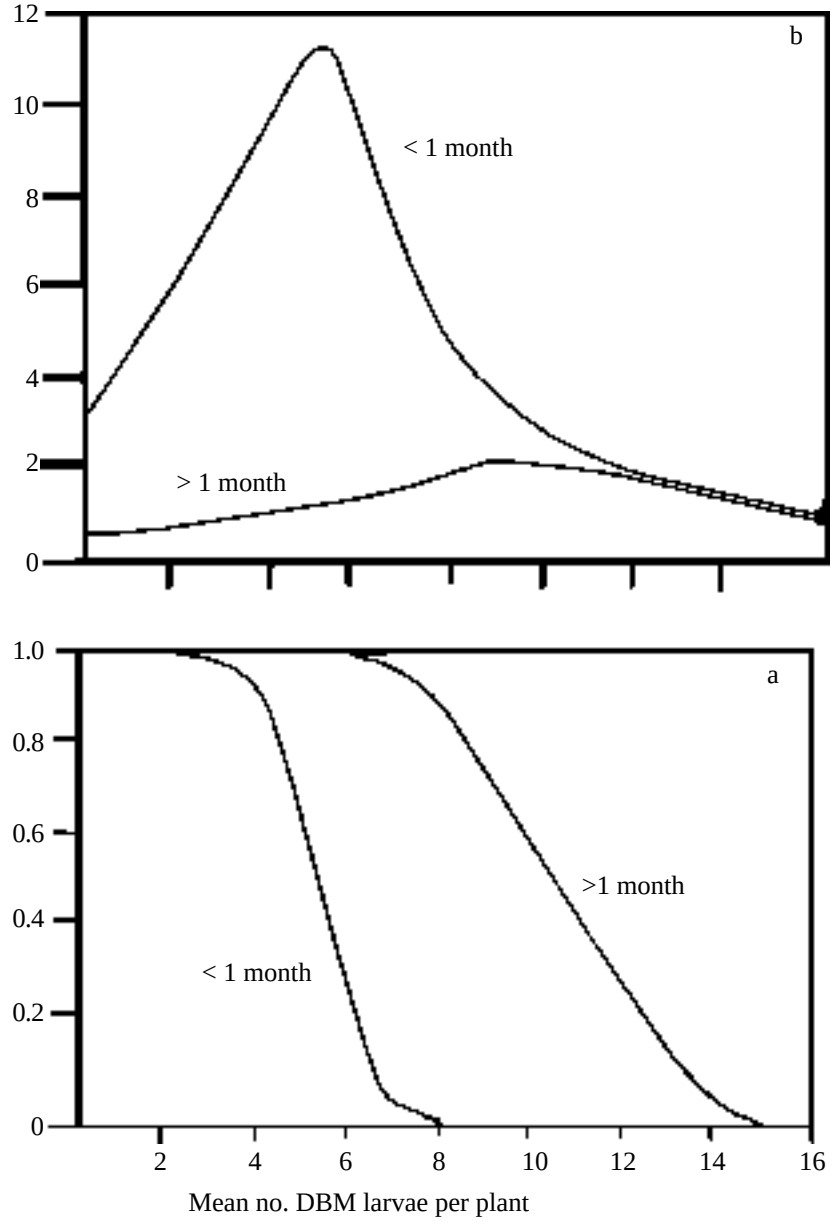


Figure 2. The operating characteristic curve for larvae of diamondback moth on cabbages for two crop ages in a sequential sampling plan.

count in the field could be compared with d_1 values. If the cumulative larvae counts exceeded d_1, then control measures should be taken. Alternatively, if the counts are less than d_0, then no action is necessary. Sampling may be continued if the counts lie between d_0 and d_1.

The probability of not recommending treatment decreases as the number of insects increases, as indicated by the operating characteristic curve *(Figure 2a)*. This is equivalent to saying that the probability of recommending treatment increases as the number of insects per plant increases.

Table 2. Sequential sampling plan in table form for field use in deciding whether to take control measures or not. Values of d_0 and d_1 are cumulative number of diamondback moth (DBM) larvae, calculated from the sequential equations: for < 1 month: $d_0 = 5.26\ n - 15.14$, $d_1 = 5.26\ n + 15.14$ and for > 1 month: $d_0 = 10.61n - 6.68$, $d_1 = 10.61n + 6.68$. If the cumulative number of larvae in the field is more than d_1, then control measures should be implemented.

	Crop age<1 month		Crop age>1 month	
Plant	d_0	d_1	d_0	d_1
1	–	20	4	17
2	–	26	15	28
3	1	31	25	39
4	6	36	36	49
5	11	41	46	60
6	16	47	57	70
7	22	52	68	81
8	27	57	78	92
9	32	62	89	102
10	37	68	99	113
11	43	73	110	123
12	48	78	121	134
13	53	84	131	145
14	59	89	142	155
15	64	94	152	166
16	69	99	163	176
17	74	105	174	187
18	80	110	184	198
19	85	115	195	208
20	90	120	206	219
25	116	147	259	272
30	143	173	312	325
35	169	199	365	378
40	195	226	418	431
45	222	252	471	484
50	248	278	524	537
55	274	304	577	590
60	300	331	630	643

From the average sample number curve *(Figure 2b)*, it is clear that very few samples will be needed when the insect populations are very low or very high. Greater number of samples are required only at intermediate density levels, *viz* more than 10 plants are needed when the number of larvae per plant is between 4–6 for crop age of less than a month.

Finally, in the comparative evaluation of the sequential sampling plan *(Table 3)*, the plan did as well as the IPM Package II, in that both determine the necessity of insecticide usage at the 6th week. However, the IPM decision was reached after sampling 60 cabbage plants, whereas the sequential sampling plan give the same conclusion after sampling one plant only. With reference to the prophylactic plot, the plan fared even better, in that action was not recommended in four times and that the decision to spray in the other three occasions was reached after sampling few plants only. Thus saving in insecticide applications amount to 4 out of 7 times, which is equivalent to 60%. On average, the need to spray in all the three plots could be detected by the 15th plant, thus cutting the sampling cost by 45/60 = 75%.

In conclusion it is obvious that there is great potential in this sequential sampling plan which could save considerable time and expense as control measures are only applied when necessary as determined by the plan. What remains to be done is to carry out a trial involving four treatments: IPM package II, prophylactic, ecological control and sequential sampling plan. We hope to do this in the near future.

Acknowledgements

We thank the Universiti Malaya and MARDI for the research facilities provided. The research is funded partly by IRPA Grant 01-02-03-0095 awarded to the senior author.

Table 3. Comparison of decisions for managing of diamondback moth (DBM) based on (a) IPM (Package II of MARDI), (b) prophylactic practices and (c) sequential sampling in three different cabbage plots in an experiment conducted in MARDI Research Station in Kelang. The DBM/plant was based on 60 plants (ie 3 replicates of 20 plants each) upon which value the IPM decision was made.

	IPM (package II) plot			Prophylactic plot			Ecological control plot		
Sample (or week)	DBM per plant	Treatment actually applied	Decision based on Seq. Samp*	DBM per plant	Treatment actually applied	Decision based on Seq. Samp.*	DBM per plant	Treatment actually applied	Decision based on Seq. Samp.*
1	0.3	none	none	0.3	spray	none	0.2	none	none
2	0.5	none	none	0.8	spray	none	0.5	none	none
3	1.3	none	none	2.1	spray	none	2.1	none	none
4	3.0	none	none	7.2	spray	spray (4, 13)	3.1	none	none
5	5.5	none	none	7.2	spray	none	5.4	none	none
6	9.4	spray	spray (1)	20.8	spray	spray (1,2,3)	9.5	none	spray (5)
7	2.6	none	none	16.0	spray	spray (1,5,7)	3.0	none	none

*Each number within the brackets indicates the number of samples (or plants) already examined when the decision that treatment was necessary was detected in a particular replicate. A total of 3 replicates were taken.

References

Ba-angood, S. A. and Stewart, R. K. (1980). Sequential sampling for cereal aphids on barley in southwestern Quebec. *Journal of Economic Entomology* ***73:*** 679–681.

Bliss, C. I. and Fisher, R. A. (1953). Fitting the negative binomial distribution to biological data and a note on the efficient fittting of the negative binomial. *Biometrics* ***9:*** 176: 200.

Chua, T.H. and Lim, B. H. (1979). Distribution pattern of diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) on choy-sum plants. *Zeitschrift fur angewandte Entomologie* ***88:*** 170–175.

Connola, D. P., Waters, W. E. and Nason, E. R. (1959). A sequential sampling plan for red-pine sawfly *Neodiprion nanulus* Schedl. *Journal of Economic Entomology* ***52:*** 600–602.

Ferrer, E. R. and Shepard, B. M. (1987). Sampling Malayan black bugs (Heteroptera: Pentatomidae) in rice. *Environmental Entomology* ***16:*** 259–263.

Fleischer, S. J. , Ravlin, F. W. and Reardon, R. C. (1991). Implementation of sequential sampling plans for gypsy moth (Lepidoptera: Lymantriidae) egg masses in eastern hardwood forests. *Journal of Economic Entomology* ***84:*** 1100–1107.

Hammond, R. B. and Pedigo, L. P. (1976). Sequential sampling plans for the green cloverworm in Iowa soybeans. *Journal of Economic Entomology* ***69:*** 181–185.

Harcourt, D. G. (1966a). Sequential sampling for us in control of the cabbage looper on cauliflower. *Journal of Economic Entomology* ***59:*** 1190–1192.

Harcourt, D. G. (1966b). Sequential sampling for the imported cabbage worm, *Pieris rapae* (L.). *Canadian entomologist* ***98:*** 741-746.

Loke, W.H., Syed Abd. Rahman, A. Sivapragasam, I. Fauziah, M. Md. Jusoh and A. K. Hussan (1996). Dynamism in diamondback moth IPM development : the Malaysian experience. *Proceedings of third international workshop on the management of diamondback moth and other curifer pests*, Kuala Lumpur.

Morris, R. F. (1954). A sequential sampling technique for spruce budworm egg surveys. *Canadian Journal of Zoology* ***32:*** 302–313.

Oakland, G. B. (1950). An application of sequential analysis to whitefish sampling. *Biometrics* ***6:*** 59–67.

Onsager, J. A. (1976). The rationale of sequential sampling, with emphasis on its use in pest management. *U.S.D.A. Technical Bulletin 1526*. 19 pp.

Pieters, E. (1978). Bibliography of sequential sampling plans for insects. *Bulletin of the Entomological Society of America* ***24:*** 372–374.

Shepard, M., Ferrer, E. R. , Kenmore, P. E., and Sumangil, J. P. (1986). Sequential sampling : planthoppers in rice. *Crop Protection* ***5:*** 319–322.

Wald, A. (1945) Sequential tests of statistical hypothesis. *Annals of Mathematical Statistics* ***16:*** 117–186.

Waters, W. E. (1955). Sequential sampling in forest insects surveys. *Forest Science* ***1:*** 68–79

Plutella equivalent action threshold for insect pests of crucifers using Chinese kale as model

Md. Jusoh Mamat
Natural Resource Management Program
Strategic, Environment & Natural Resource Center
MARDI, Serdang, Selangor.

Abstract

An experiment was conducted in June 1995 at MARDI Research Station in Serdang to evaluate the effectiveness and practicality of the *Plutella* Equivalent (PE) action threshold in comparison to the Current IPM 1 and the Modified IPM 2 action thresholds in deciding whether to spray or not to spray insecticide against insect pests of Chinese kale (*Brassica oleracea var. alboglabra).* The treatments were arranged in an RCBD and consisted of 3 thresholds (Current IPM 1, Modified IPM 2 and PE) and an untreated plot. Both Current IPM 1 and Modified IPM 2 action threshold are based on *Plutella xylostella* and *Hellula undalis* populations as well as the percentage parasitism of *P. xylostella.* The PE action threshold however, is based on total population of all species, each of which is weighted to *P. xylostella* where 1 *H. undalis or Crocidolomia binotalis* = 4 PE, 1 *Spodoptera litura* 3&4 instar = 2 PE and 1&2 instar = 1 PE and 1 flea beetle = 1 PE. The results showed that the PE action threshold is sensitive, effective and more practical than the Current IPM 1 or the Modified IPM 2 as a decision criteria to spray or not to spray for insect pests of Chinese kale although no significant difference was recorded between them in terms of mean number of insect pests per plant, mean percentage leaf damage at harvest, mean yield and mean net return per ha.

Key words: *Plutella,* action threshold, IPM, Malaysia

Introduction

The search for an effective Integrated Pest Management package for diamondback moth on crucifers started as early as the mid 1970s (Yusof & Lim, 1992). However, only in 1988 the full package was introduced to and practiced by some cabbage growers of the Cameron Highlands. The package was developed by incorporating a three-tiered economic threshold level (Loke *et al.,* 1990) which was based on the original threshold of 37 larvae/10 plants (Md. Jusoh *et al.,* 1982) and emphasizing the use of biological control agents such as *Bacillus thuringiensis* Berliner, *Cotesia plutellae* Kurdjumov, *Diadegma semiclausum* Horstmann and *Diadromus collaris* Gravenhorst. The present adopted threshold was considered by many to be too cumbersome to be practiced by the farmers and become unreliable when multiple species infestation occurs (Shelton *et al.,* 1982) which is often the case in Malaysia where *Hellula undalis*, *Spodoptera litura*, *Crocidolomia binotalis* and flea beetles are found infesting at the same time in a single crop. Thus, this paper is proposing an alternative threshold, the *Plutella* equivalents (PE) action threshold.

Materials and Methods

The experiment was conducted in June 1995 at MARDI Research Station in Serdang. A 40 m by 9 m plot was divided into 4 replication blocks (9.15 m by 9 m each) and planted with chinese kale (local variety) on June 6, 1995 using 4 week old seedlings. Each block was further subdivided into 4 treatment plots where each plot is made up of 3, 9.15 m by 0.76 m beds. Each bed contains 120 plants planted in two rows at planting distance of 15 cm between plants and 46 cm between rows. The treatments were arranged in an RCBD and consisted of 3 thresholds (Current IPM 1, Modified IPM 2 and PE) and an untreated control plot *(Table 1).* Both, Current IPM 1 and Modified IPM 2 action thresholds are based on *P. xylostella* and *H. undalis populations*, as well as the percentage parasitism of *P. xylostella.* In Current IPM 1 action threshold, a decision to spray with *B. thuringiensis* var *aizawai* (Florbac®) is made when the population of *P. xylostella* larvae is between 4 to 7 per plant and the percentage parasitism is less then 40% . A decision to spray with acephate (Orthene®) is made when *P. xylostella* larvae population exceeded 7 per plant. For Modified IPM 2, the action threshold for *P. xylostella* in the second month onward is increased to 8 to 14 and >14 larvae, respectively. In the case of *H. undalis* for both action thresholds, a decision to do the shoot treatment with *B.t* (Florbac®) is made when one larvae per plant is present. In PE action threshold, a decision to spray with *B.t* (Florbac®) is made when the population exceeded 2 per plant (where 1 *H. undalis or Crocidolomia binotalis* = 4 PE, 1 *Spodoptera litura* 3&4 instar = 2 PE and 1&2 instar = 1 PE, 1 flea beetle = 1 PE) and the lepidopterans constitute more than 70% of the pest numbers. If <70% lepidopterans, acephate (Orthene®) is used instead. Insect counts were made on 20 plants per plot chosen at random on a weekly interval. Percentage leaf damage was recorded on 20 plants per plot and yield (total green weight)

were taken at harvest. The net return per hectare was calculated based on current Chinese kale market price of RM 2.00 per kg and total cost of production (excluding the cost of insecticide spray) at RM 1,625.00 per hectare crop. All data were subjected to ANOVA and the Duncan's New Multiple-Range Test for treatment means separation.

Results and Discussion

The effects of the three action thresholds on insect pest populations are shown in *Figure 1*. Neither the *P. xylostella* nor the *Plutella* Equivalent (PE) population counts exceeded the threshold level of 80 larvae/20 plants in both of the treatments, i.e. the Current IPM1 (T1) and the Modified IPM2 (T2) action thresholds. However, *H. undalis* was present at least one larva/20 plants on the first and second week after planting which resulted in the decision to spray with Florbac® as shoot treatments. In the case of PE action threshold treatment (T3), the threshold of 40 PE/20 plants was exceeded only during the first week, which triggers the decision to spray with Orthene®. For the untreated control (T4), the PE population exceeded the PE threshold of 40 larvae/20 plants during the first, second and third week of the crop period. The current IPM 1 threshold for *P. xylostella* of 80 larvae/20 plants was exceeded only in the second and third week of the crop period.

The mean number of insect pests in terms of PE showed no significant difference between the three threshold treatments, but are significantly different to the untreated control which is about 4–8 folds higher *(Table 2)*. Similar results were obtained for the mean percentage leaf damage, mean yield and the mean net return where the untreated control resulted in a RM767 loss. These results seem to indicate that for a short term crop like Chinese kale which are likely to be moderately infested by more than one pest species, action thresholds that are based on *P. xylostella* population alone will not likely to exceed the threshold level of 80 larvae/20 plants throughout the Chinese kale cropping period which is only 3–4 weeks, but the resulting yield loss could be significant. The net returns shown in *Table 2* may be over-estimated since the cosmetic effects of leaf damage was not taken into

Table 1. Action thresholds utilized in the experiment conducted at MARDI Research Station in Serdang, June 1995

Action thresholds	Criteria	Decisions
1. Current IPM 1	DBM:	
	< 4 larvae/plant	No spray
	> 4 < 7 & parasitization > 40%	No spray
	> 4 < 7 & parasitization < 40%	Spray with Florbac®
	> 7 larvae/plant	Spray with Orthene®
	Hellula:	
	1 larva/20 plant or more	Shoot treatment with Florbac®
2. Modified IPM 2	DBM:	
	< 8 larvae/plant	No spray
	> 8 < 14 & parasitization > 40%	No spray
	> 8 < 14 & parasitization < 40%	Spray with Florbac®
	> 14 larvae/plant	Spray with Orthene®
	Hellula:	
	1 larva/20 plant or more	Shoot treatment with Florbac®
3. *Plutella* Equivalent (PE)	2 or higher & lepidopterans > 70%	Spray with Florbac®
	2 or higher & lepidopterans < 70%	Spray with Orthene®
	Where:	
	1 *Hellula*/1 *Crocidolomia* larva = 4 PE	
	1 *Spodoptera* 3 & 4 instar larva = 2 PE	
	1 *Spodoptera* 1 & 2 instar larva = 1 PE	
	1 Flea beetle adult = 1 PE	

Table 2. Effects of various action thresholds on insect pest numbers, yield and net returns of Chinese kale, MARDI Research Station, Serdang, June 1995

Treatments	*Mean no. of insect pest (PE/20 plants)	*Mean leaf damage at harvest (%)	*Mean yield (kg/ha)	Total no. of sprays	Total spray cost (RM/ha)	*Mean Net return (RM/ha[1/])
Current IPM	9.42a	4.75a	1236a	2	279.00	568.00a
Modified IPM 2	12.75a	5.88a	1425a	2	279.00	946.00a
Plutella Equivalent	17.17a	5.50a	1404a	1	157.00	1026.00a
Control (untreated)	81.92b	50.81b	429b	0	0	(767.00b)

*Means follwed by the same letter are not significantly (5%) different by DNMR test.

[1/]Calculated based on Chinese kale price of RM 2.00/kg and a total cost of production (excluding cost of insecticides spray) at RM1625.00/ha. crop.

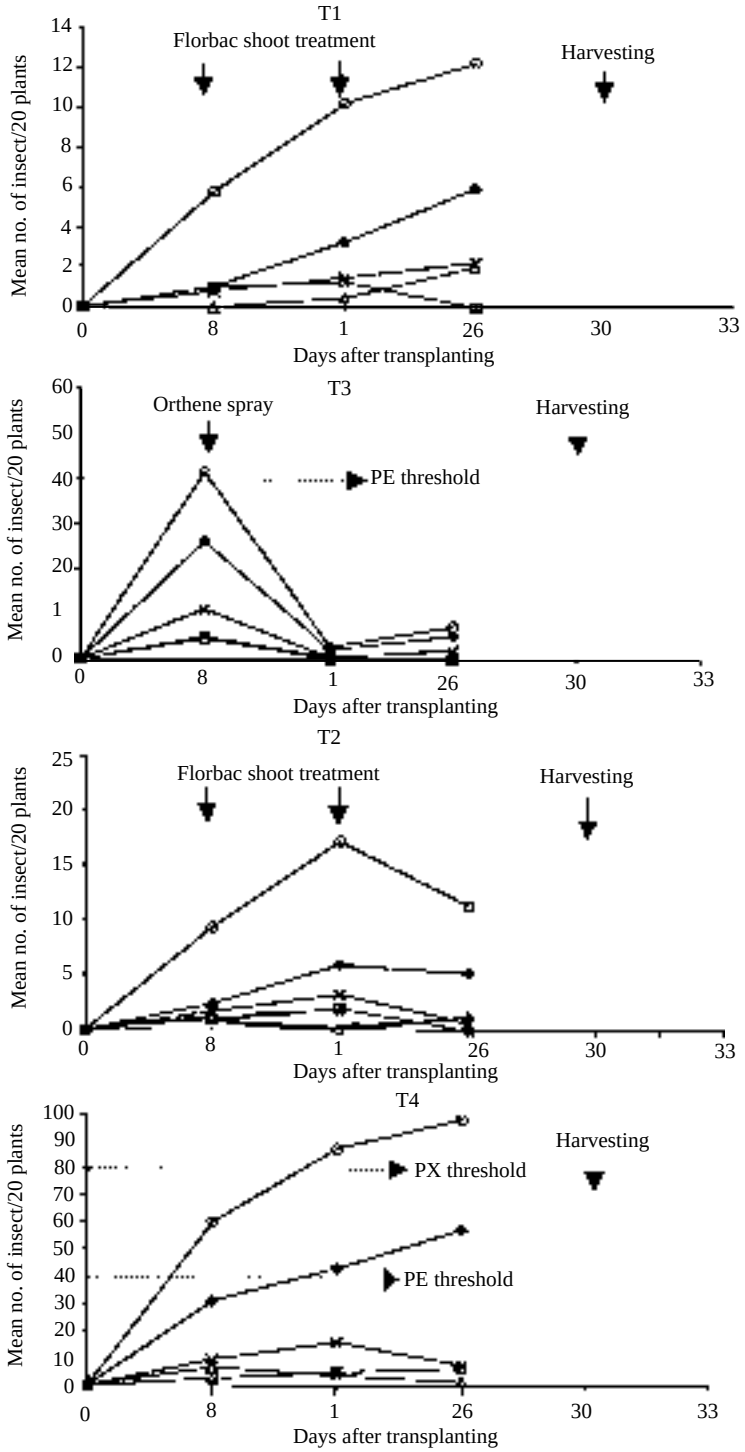


Figure 1. Effects of various thresholds (T 1 = Current IPM, T 2 = Modified IPM 2, T 3 = Plutella equivalent and T 4 = untreated control) on insect pest populations infesting Chinese kale at MARDI Station Serdang, June 1995.
(❑ Plutella xylostella: ◆ Hellula undalis; Δ Spodoptera litura; X Flea beetle; O Plutella equivalent).

account. Nevertheless, the PE action threshold proved to be effective and more practical compared to the other two action thresholds.

Acknowledgements
The author is grateful to his research assistants, Mr. Muhamad Shah Ab. Lah and Mr. Ahmad Basir for their active involvement in field experiments; to Director General of MARDI for his permission to present this paper in the Third International Workshop on The Management of Diamondback Moth and Other Crucifer Pests

References

Loke, W. H., Lim, G. S., Syed, A. R., Abdul Aziz, A. M., Rani, M. Y., Md. Jusoh, M., Cheah, U.B. and I. Fauziah. (1992). Management of diamondback moth in Malaysia: development, implementation and impact. pp. 529–540. *In: Diamondback Moth and Other Crucifer Pests – Proceedings of the Second*

International Workshop, Tainan, Taiwan, 10–14 December 1990 (ed. by N.S. Talekar). AVRDC, Tainan, Taiwan.

Md. Jusoh, M., Heong, K.L. and G. S. Lim. (1982). Assessment of cabbage losses inflicted by the diamondback moth in Peninsular Malaysia. *MARDI Res. Bull., (1982) 10,* ***2:*** 186–194.

Shelton, A. M., Sears, M. K., Wyman, A. J. and T. C. Quick. (1983). Comparison of action thresholds for lepidopterous larvae on fresh-market cabbage. *J. Econ. Entomol. Vol. 76, no. 1: 196–199.*

Yusof, O. and B. K. Lim. (1992). Diamondback moth in Malaysia. pp.1–3. *In: Training Manual on Integrated Pest Management of Diamondback Moth in Cabbage in Malaysia (eds. Md. Jusoh, M., W. H. Loke, A. R. Syed and Mac Tyre).* MARDI, Kuala Lumpur, Malaysia.

Control of lepidopteran insects in leafy cole crops with spinosad insect control agent

R.W. Naish[1], K. Kaneshi[2] & D. Sunindyo[3]

[1]*DowElanco Pacific Limited c/o P.O.Box 8394/JKS, Jakarta, 12083 Indonesia*

[2]*DowElanco Division Dow Chemical Japan Ltd.,12/F Tennoz Central Tower, Shinagawa-ku,Tokyo, Japan.*

[3]*PT Pacific Chemicals Indonesia, P.O.Box 8394/JKS, Jakarta, 12083 Indonesia*

Abstract

SUCCESS* (ISO proposed common name spinosad) is the first insect control agent in the NATURALYTE* class of naturally derived products under development at DowElanco. Field trials undertaken on leafy cole crops (cabbage, Chinese kale and broccoli) in several Asian countries are reported. Results demonstrate excellent activity on diamondback moth, *Plutella xylostella* (L), cabbage cluster caterpillar, *Crocidolomia pavonana* (Fabricius), cabbage white butterfly, *Pieris rapae* (Linnaeus) and moderate to good activity on beet army worm *Spodoptera exigua* (Hubner). Laboratory studies confirmed no cross resistance to methyl parathion, fenvalerate, teflubenzuron and abamectin resistant strains of diamondback moth. A laboratory study also demonstrated safety to *Diadegma semiclausum* (Hellen) which together with its excellent ecotoxicological and environmental profiles confirmed suitability of spinosad for use in integrated pest management programs.

Key words: Diamondback moth, spinosad, cole crops, *Diadegma semiclausum*, integrated pest management (IPM)

Introduction

Management of insect pests in crops today presents many challenges. Occurrence of insect resistance makes it desirable to discover management tools with a new mode of action; the increasing sophistication of integrated pest management requires compounds with favourable environmental profiles and selectivity to beneficial arthropods; reduced risk afforded by lowered use rates together with greater mammalian and human safety are always overarching considerations in new product discovery effort. Fermentation products have the potential to deliver many if not all these unique advantages. This paper will introduce and describe some of the features and potential uses of spinosad (ISO proposed common name) the first of the NATURALYTES*, a structurally unique new class of fermentation derived tetracyclic macrolides for insect management from DowElanco.

Spinosad (brand name SUCCESS*), belongs to the family of naturally derived compounds called spinosyns, which are the products of fermentation by a novel actinomycete which has been classified as *Saccharopolyspora spinosa.* This organism was first isolated by Eli Lilly and Company scientists from a soil sample taken from a Carribean island in 1982, and by 1985 its fermentation broth had been shown to be active on mosquito larvae and armyworms. By 1988 milligram quantities of the isolated spinosyns A and D were produced. These two of more than 20 factors produced by *S. spinosa* are among the most active as insect control agents, and spinosad (formerly XDE-105) is the proposed common name for an 85:15 percent ratio of spinosyns A and D.

*SUCCESS and NATURALYTE are Trademarks of Dow Elanco

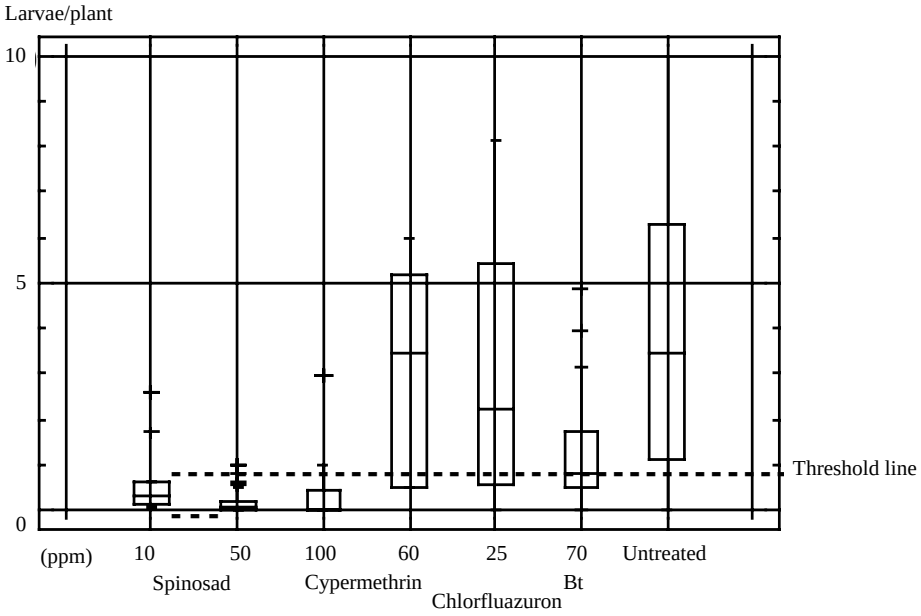
The chemical, physical and biological properties of spinosad have been reviewed (Anonymous, 1993). Spinosyns A and D are similar in their chemical and biological behaviour. They are short lived in the environment, having soil half lives of 9–17 days, and persistence on plant and soil surfaces are of the order of a few days. Soil sorption is moderately strong and their rapid soil degradative routes limit potential to move to surface and ground waters. The acute toxicity of spinosad is also low with acute oral LD_{50} in rats of >3 700 mg/kg and dermal LD_{50} in rabbit of >2 000 mg/kg. Avian and fish toxicity are such that spinosad can be considered practically non-toxic.

Spinosad is principally active on the order Lepidoptera, but it is also very effective against species in several other insect orders including Diptera, Hymenoptera, Isoptera and Thysanoptera. The compound is effective both by contact and orally, the latter appearing to be the primary route in most species. To date the exact mode of action is not known; it appears to be unique, in that it acts on the insect nervous system in a manner that is different from all other known pest control products. This unique mode of action will provide for increased options for integrated pest management and will offer the farmer a new material to rotate within resistance management programs.

Asia-Pacific field testing

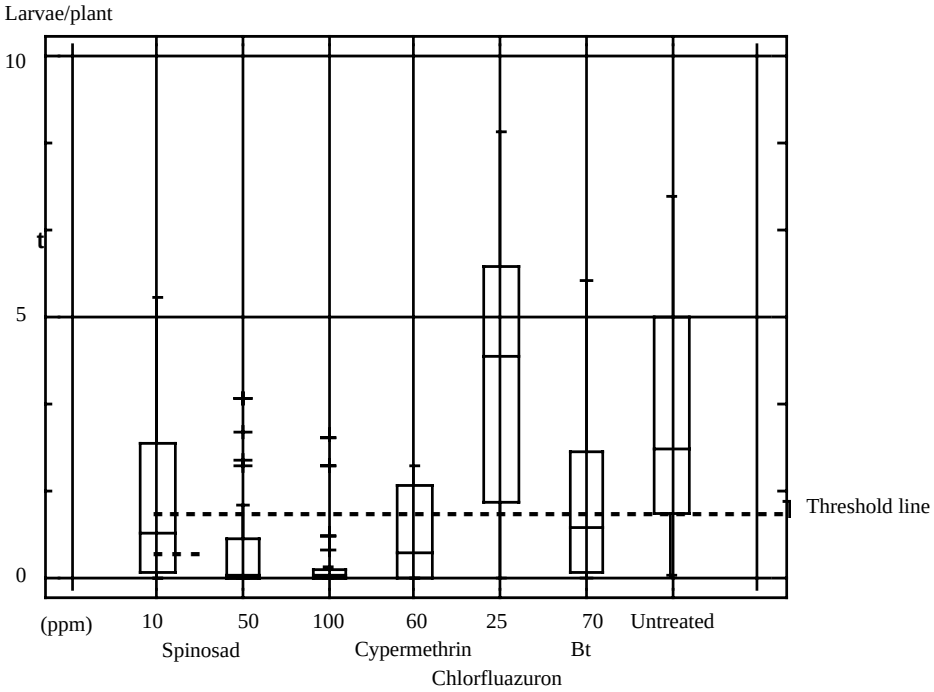
Japan

Fifty field trials were conducted during 1995–96 with spinosad (25%WG formulation) to evaluate control of lepidopteran insects on cabbage *Brassica oleracea* (L), Chinese cabbage *B. chinensis* (L) and Japanese radish *Raphanus sativus* (L) at DowElanco field stations, or



Results from 49 Trials – Japan 1994 & 95

Figure 1. Efficacy of spinosad (25WG) on Plutella xylostella (DBM) – 7 Days after application –



Results from 39 Trials – Japan 1994 & 95

Figure 2. Efficacy of spinosad (25WG) on Pieris rapae (CWB) – 7 days after application –

by outside co-operators and local government officers (official trials). Trials were conducted following local cultural practice and application methodology. Average spray volume was 1 900 L/ha, though the range was 500–3 200 L/ha depending on crop, growth stage, plant density and type of equipment.

Data from cross-trial analysis of the 46 trials with diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) infestations are presented in *Figure 1*. Rates of spinosad as low as 12.5 g ai/ha (6.25 ppm) provided acceptable control of diamond back moth but a rate of 37.5–50 g ai/ha (25 ppm) was required for highly consistent reduction of insects below the threshold level of one larva per head at onset of head formation. Performance of spinosad was much more reliable than the commercial standards, Bt, cypermethrin and chlorfluazuron.

Data from cross-trial analysis of the 38 trials with cabbage white butterfly *Pieris rapae* (Linnaeus)(Lepidoptera: Pieridae) infestations is presented in *Figure 2*. Compared to diamondback moth, cabbage white butterfly susceptibility to spinosad was much more variable. Although the mean larval number across trials for the 50 g ai/ha (25 ppm) rate of spinosad was below the industry threshold, within trial variability was greater than for some of the industry standards. A rate of 75–100 g ai./ha (50 ppm) spinosad provided a much more consistent

result and was equal or better than the current industry standards.

Thailand

Five trials were conducted between 1994 and 1996, three investigating length of residual effectiveness of spinosad (as a 48%SC formulation) on diamondback moth and beet armyworm *Spodoptera exigua* (Hubner) (Noctuidae: Lepidoptera) in Chinese kale *Brassica alboglabra* following a single spray application, and two (one in cabbage *Brassica oleracea*, one in Chinese kale) assessing control of diamondback moth in a multiple spray program with applications at approximately weekly intervals. All spray treatments were made in spray volumes of 1 000 L/ha applied with a knapsack sprayer or motorcycle sprayer.

Mean percent control of diamondback moth across the three trials demonstrating residual effectiveness of spinosad are presented in *Figure 3*. Spinosad 50 g ai/ha was required to provide control of diamondback moth equivalent to the commercial standards, abamectin 18 g ai/ha or fipronil 50 g ai/ha. All three treatments provided good residual activity on this crop for up to five days, but declined significantly by seven days after application. For control of beet armyworm *(Figure 4)* 50–100 g ai/ha of spinosad was required for acceptable control, while of the three standards, chlorphenapyr, abamectin and fipronil, only chlorphenapyr showed acceptable activity on this pest at the rates tested. Residual effect of these treatments was evident for 10 days, thereafter the pest pressure declined rendering further assessment meaningless.

Figure 5 shows mean percent diamondback moth control data calculated from four assessments, in one trial on Chinese kale and five in the other on cabbage, made at four and seven days after each spray application in the multiple spray trials. Again a rate of 50g ai/ha of spinosad was required to provide control equivalent to the best of the commercial standards, abamectin and chlorphenapyr. The poor control obtained with deltamethrin and chlorfluazuron (data not included in *Figure 5*) was assumed to be due to development of resistance by diamondback moth to these insecticides.

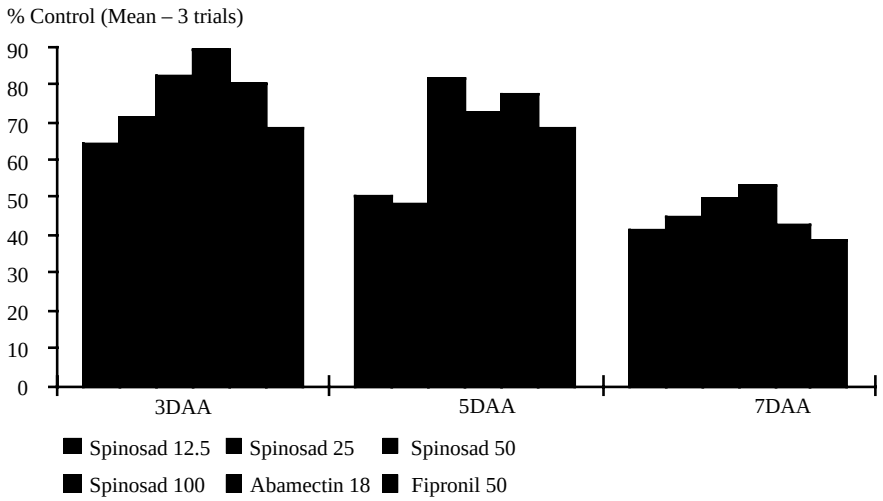


Figure 3. Residual effect of spinosad on diamondback moth in Chinese kale – Thailand 1994–5

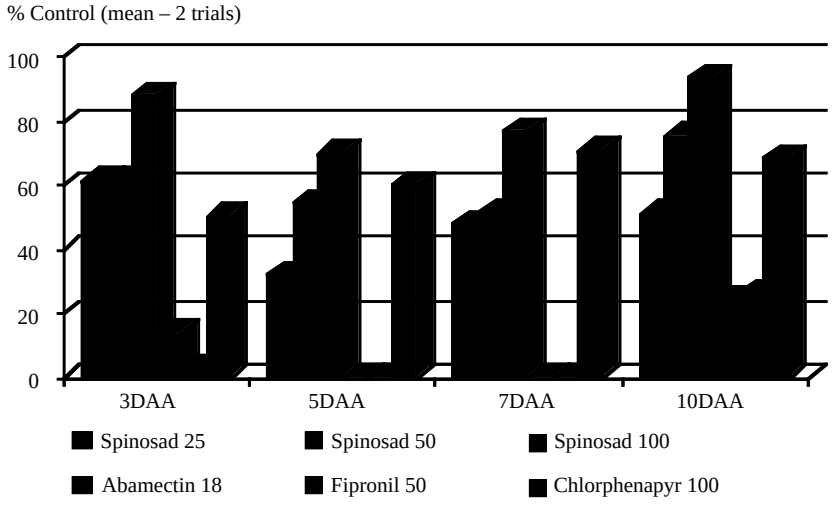


Figure 4. Residual effect of spinosad on beet armyworm in Chinese kale – Thailand 1995

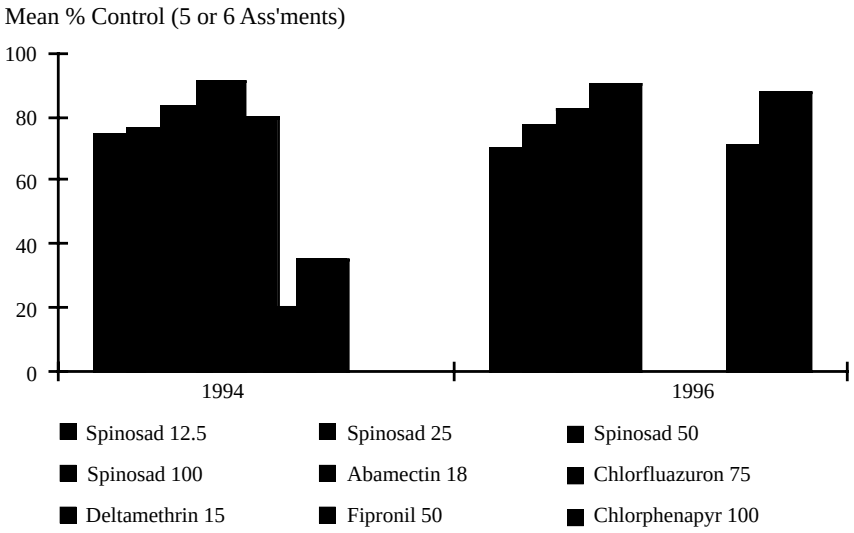


Figure 5. Spinosad multiple spray programs for DBM control – Thailand

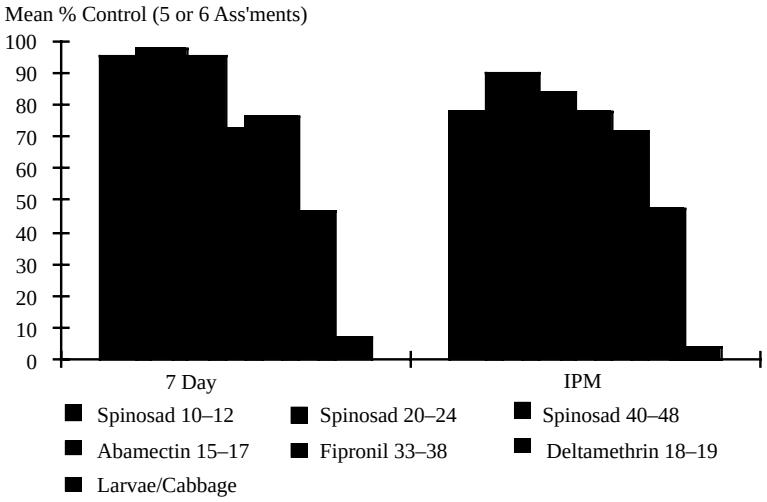


Figure 6. Comparison of weekly and IPM spray schedules with spinosad on DBM

Indonesia

Four of six trials conducted in Lembang and Malang between 1993–1996, are reported. All were season long spray program trials; two with applications at weekly intervals, and the others with timing of repeat sprays when the IPM spray threshold (one larva per two plants) was met or exceeded. In all cases spray application was made with hand knapsack sprayers with spray volumes varying from 400 L/ha early in the season to 1 200 L/ha at crop maturity. Spray applications were made following local practice of using the same dilution at each spray application and increasing the total spray volume according to the stage of the crop. However for ease of comparison, each dose rate was reported in terms of the average use rate (g ai/ha) over the duration of the trial.

Mean percent control of diamondback moth from the two sets of trials are summarised in *Figure 6*. Data are the means of six assessments from trials conducted at Malang or nine assessments from trials conducted at Lembang. Insecticide rate ranges are shown because of the different number of sprays and spray volumes at each location; six sprays at Malang (average spray volume 650 L/ha) and nine at Lembang (average spray volume 767 L/ha). Spinosad (as a 2.5% SC formulation) at the lowest rate reported, 10–12 g. ai/ha provided a higher level of control than the comparative standards in the weekly programs and equalled the same standards in the IPM programs. Deltamethrin in either program failed to provide adequate control presumably due to diamondback moth resistance. Cabbage cluster caterpillar, *Crocidolomia pavonana* (Fabricius), (Lepidoptera; Pyralidae) was present in all trials and was completely controlled by all rates of spinosad and by all of the other treatments except deltamethrin

The overall level of control of diamondback moth with all insecticide treatments was higher in the weekly spray programs than in the IPM programs; control levels with the latter on average 10 percent lower. This resulted in lower yields of marketable heads of cabbage in the Malang trial, but the converse was seen at the Lembang trial. It was also observed that the overall use of insecticides was reduced from an average of nine sprays at Lembang with the weekly program to four with the older type insecticides, and as few as two with spinosad 40–48 g ai/ha or abamectin 15–18 g ai/ha when the IPM threshold program was followed.

A corresponding reduction in spray number was also found at Malang.

Laboratory studies

Speed of knockdown

In a laboratory study conducted at the DowElanco field station in Ping Tung, Taiwan, speed of knockdown and extent of feeding of diamondback moth after exposure to spinosad was determined after either direct contact exposure, or ingestion of treated cabbage discs (stomach poisoning). Data is summarised in *Table 1*, and shows that ingestion was the primary route of intoxication, with 100% mortality in 48–72 hours with doses approximating field use rates of spinosad, and that feeding was arrested in three hours following ingestion or about 24 hours if exposure was by the contact route only.

Cross-resistance studies

Sun (1991 and 1996) compared by laboratory topical spray application to fourth instar larvae, the susceptibility of a number of resistant strains of diamondback moth to spinosyn A (the main insecticidal factor in spinosad). She found that methyl parathion, fenvalerate, chlorfluazuron and abamectin resistant strains were all susceptible to spinosyn A (small apparent resistance ratios within strains were observed, but were considered typical of this type of study) whereas all strains except methyl parathion-R were clearly cross resistant to cypermethrin *(Figure 7)*. In subsequent studies, she and her co-workers determined that the main isozymes involved in the detoxification of these insecticides in the diamondback moth strains tested were GST-3, GST-4 and P-450, and concluded from the lack of cross resistance that spinosyn A appeared unaffected by any of these isozymes.

Beneficial insects study

Setiawati of the Entomological Society of Indonesia, working at Lembang, (1995) tested the selectivity of spinosad and several other insecticides to *Diadegma semiclausum* (Hellen) (Hymenoptera: Ichneumonidae) using a larval dipping procedure with measurement of mortality of the parasite relative to mortality of second and fourth instar diamond back moth larvae 72 hours post-treatment. Data from these tests *(Table 2)* demonstrated that spinosad would be unlikely to impact *D. semiclausum* at spray application rates required to control diamondback moth and cabbage cluster caterpillar under Indonesian conditions.

Conclusion

The series of trials reported demonstrated excellent efficacy of spinosad on diamondback moth and other lepidopteran insects commonly occurring in cole crops in Asia. There was quite a wide variation in the rate required for a high level of control in different

Table 1. Comparison of contact and ingestion toxicity of spinosad to diamondback moth

	Time	3 hours	12 hours	24 hours	48 hours
% Mortality*	Topical	13	33	36	40
	Ingestion	0	67	77	100
% Leaf consumption*	Topical	0.2	0.5	4.0	0
	Ingestion	0.1	0	0	0

*Spinosad rate of 40 ppm as topical application to 3rd instar larvae or as cabbage leaf disc dip.

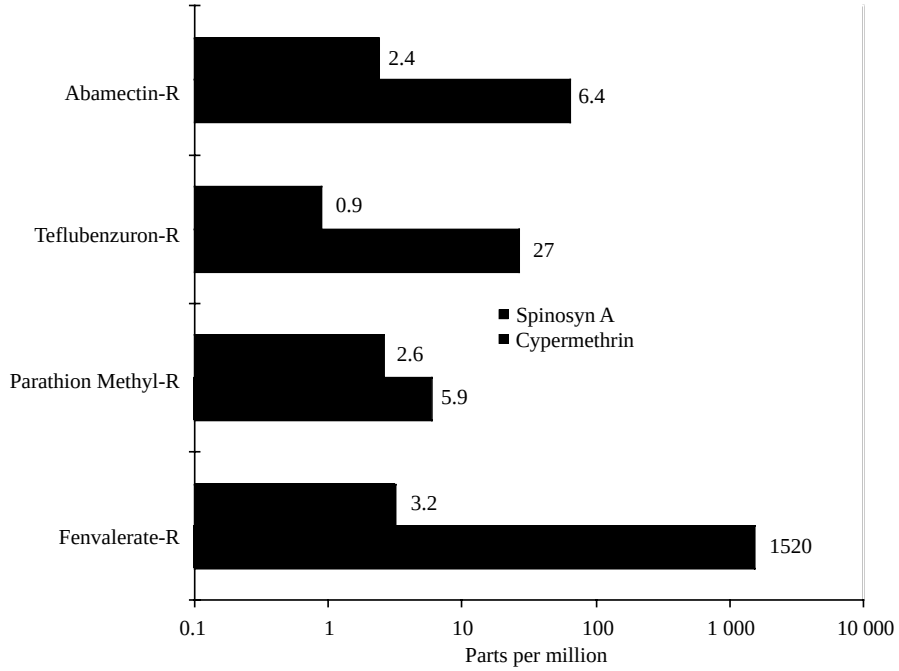


Figure 7. Resistance ratios of diamondback moth utilising resistant strains

Table 2. Toxicity of insecticides to diamondback moth and *Diadegma* (Laboratory dip test)

Test substance*	Diamondback Moth (2nd instar) 72 hr LC_{50}	Diamondback Moth (4th instar) 72 hr LC_{50}	*Diadegma* sp. 72 hr LC_{50}	Selectivity ratio between 4th instar DBM & *Diadegma***
Spinosad	0.91	1.91	28.23	14.8
Fipronil	5.76	2.5	1.68	0.7
Abamectin	0.8	12.04	3 656	303.6
B. thuringiensis	1 422	3 682	13 215	3.6

*Test substances tested as formulated products

**Selectivity ratio of 1–10 = low selectivity; above 10 = good selectivity

geographic areas. Further trials will be required to fully elucidate the reasons for these variations, and to fine tune commercial use rates for each country in the region, as well as to integrate use of spinosad in a workable insect resistance management program. Overall spinosad has very favourable toxicology and regulatory attributes. These include reduced environmental load, reduced risk to beneficial insects, minimal concerns for groundwater contamination, unique mode of action, no known resistance or cross resistance, excellent efficacy and increased options for integrated pest management programs.

Acknowledgement

The authors gratefully acknowledge the assistance of Dr. Chih-Ning Sun, Professor of Insect Toxicology, National Chung Hsing University, Taichung, Taiwan for the studies on diamondback moth cross resistance; Dr. Wiwin Setiawati at Balai Penelitian Sayuran, Lembang, Indonesia for the field trial work on diamondback moth and the study on selectivity to *Diadegma sp*.; Dr. M. Suria, at Balai Penelitian Tlekung, for the field trials at Malang; Dr.Winai Rushtapakornchai, Entomology and Zoology Division, Dept. of Agriculture Bangkok, for the Thailand field trials, and the Research staff of DowElanco in the Pacific area for their assistance with the research reported.

References

Anonymous (1993). Spinosad Technical Guide. 25 pages. Indianapolis: DowElanco

Sun, C.N. (1991 & 1996). Personal communication, Department of Entomology, National Chung Hsing University, Taichung, Taiwan.

Setiawati,W (1995). Personal communication, Entomological Society of Indonesia, Balai Penelitian Sayuran, Lembang, Indonesia.

Emamectin benzoate: a novel avermectin derivative for control of lepidopterous pests

R. K. Jansson, R. Brown, B. Cartwright, D. Cox, D. M. Dunbar, R. A. Dybas,[1] C. Eckel, J. A. Lasota, P. K. Mookerjee, J. A. Norton, R. F. Peterson, V. R. Starner and S. White

Merck Research Laboratories, Agricultural Research and Development, Merck & Co., Inc., P.O. Box 450, Hillsborough Road Three Bridges, NJ 08887 U.S.A.
[1]Current Address: 298 Reaville Rd., Flemington, NJ 08822

Abstract

Emamectin benzoate (MK-0244) is a novel semi-synthetic derivative of the natural product abamectin in the avermectin family of 16-membered macrocylic lactones. This epi-methyl amino derivative has unprecedented potency against a broad spectrum of lepidopterous pests with LC_{90}values ranging between 0.001–0.02 *ug*/ml in ingestion-based foliar spray assays. Emamectin benzoate is ca. 1,500-fold more potent against certain armyworm species, and 2- to 5-fold more potent against diamondback moth, *Plutella xylostella* (L.), than abamectin. It is 18- to 80,400-fold more potent against *P. xylostella*, cabbage looper, *Trichoplusia ni* (Hubner), and beet armyworm, *Spodoptera exigua* (Hubner), than other new insecticides, such as fipronil, chlorfenapyr, and tebufenozide. In the field, the compound is very effective at controlling all lepidopterous pests of cole crops at low use rates (8.4 g ai/ha). The mode of action is similar to abamectin (GABA - and glutamate-gated chloride channel agonist) and is not cross resistant with any other compound currently used commercially. The first registrations for the compound on cole crops in the U.S. and Japan are anticipated for 1997. An overview of its potential for control of lepidopterous pests in cole crops is provided.

Key words: Emamectin benzoate, avermectin derivative, lepidoptera, cole crops

Introduction

Avermectins are a family of 16-membered macrocyclic lactone natural product homologues produced by the soil microorganisms, *Streptomyces avermitilis* MA-4680 (NRRL 8165) and were isolated at Merck Research Laboratories from a soil sample collected in Japan by researchers at the Kitasato Institute (see Campbell, 1989 and references therein). Isolation of the crude fermentation product of *S. avermitilis* yielded a complex of eight closely related avermectin homologues (A_{1a}, A_{1b}, A_{2a}, A_{2b}, B_{1a}, B_{1b}, B_{2a}, and B_{2b}), of which avermectins B_1 (a and b) were the major components. Abamectin, the non proprietary name assigned to avermectin B_1, is a mixture of B_{1a} (≥80%) and B_{1b} (≤20%). This mixture was very potent against mites and certain insect species (Dybas *et al*., 1989). Abamectin was developed for crop protection and is currently sold commercially for control of mites and certain insect pests on several ornamental and horticultural crops in over 50 countries. Ivermectin, a 22, 23 dihydro semi-synthetic derivative of abamectin, was developed widely for control of ecto- and endoparasites of food and companion animals as well as for control of the causative agent of river blindness, *Onchocerca volvulus*, in man (see Campbell, 1989; Lariviere *et al*., 1985).

Although abamectin was potent against mites and a select number of insects, it was considerably less potent against most Lepidoptera. This spectrum deficiency prompted a focused, medicinal chemistry and biological testing program that resulted in the discovery of 4"-epi-methylamino-4"-deoxyavermectin B_1 (emamectin) in 1984 *(Figure 1)*. MK-0243 (the hydrochloride salt of emamectin), which was derived from abamectin via a five-step synthesis (Cvetovich *et al*., 1994), was discovered after screening several hundred avermectin derivatives in an *in vivo* screen using tobacco budworm, *Heliothis virescens* (Guenee), and southern armyworm. *Spodoptera eridania* (Cramer) (Dybas and Babu, 1988; Dybas *et al*., 1989; Mrozik, 1994; Mrozik *et al*., 1989). The benzoate salt of emamectin (coded MK-0244) had improved thermal stability and greater water solubility compared with the hydrochloride salt. MK-0244 was assigned the nonproprietary name emamectin benzoate and is currently being developed as a crop protection insecticide; first registrations in the U.S. and Japan are anticipated for 1997. The present paper presents an overview of the potential of emamectin benzoate for control of lepidopterous pests of cruciferous crops.

Mode of action

The mode of action of the avermectins has been reviewed by several authors (Arena, 1994; Fisher and Mrozik, 1984; Rohrer and Arena, 1995; Turner and

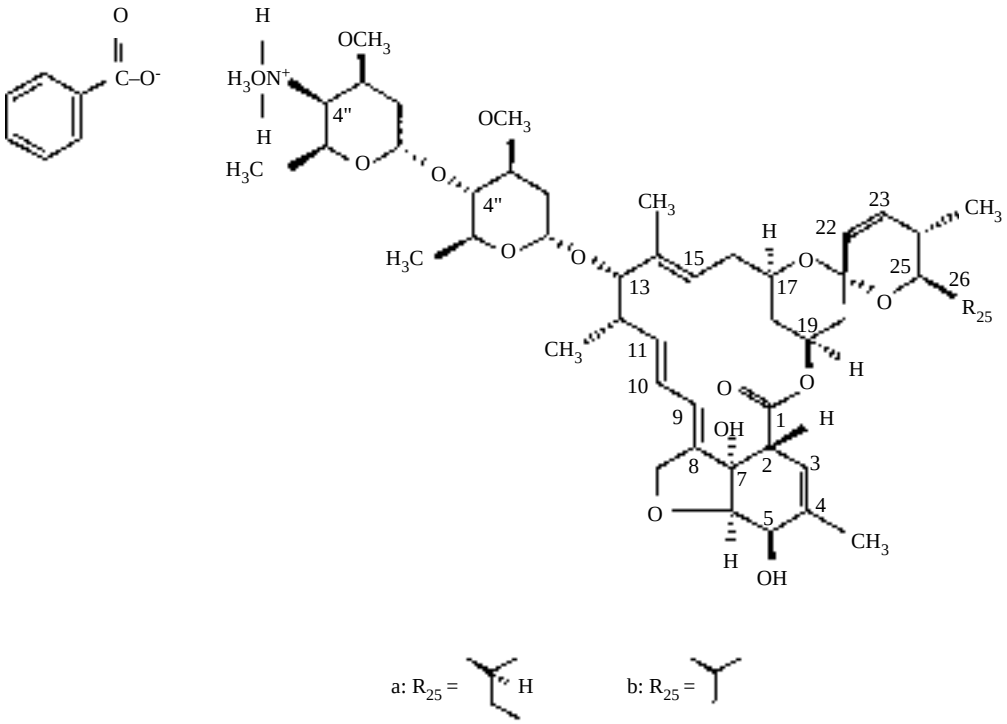


Figure 1. Structure of emamectin benzoate (MK–0244)

Schaeffer, 1989). All studies suggested that there are few qualitative differences in the mode of action of the avermectin compounds studied, including emamectin benzoate; thus, it is believed that most, if not all, avermectins have a similar mode of action.

The anthelmintic properties of the avermectins are due predominantly to potentiation and/or direct opening of glutamate-gated chloride channels, whereas in insects, it is likely that avermectins bind to multiple sites (including glutamate and GABA) in insect chloride channels. In general, the chloride ion flux produced by the opening of the channel into neuronal cells results in loss of cell function and disruption of nerve impluses. Consequently, invertebrates are paralyzed irreversibly and stop feeding. Maximum mortality of arthropods is achieved within 4 days. Although the avermectins do not exhibit rapid knock down activity against insects, paralysis is rapid, and feeding damage to crops is minimal because insects cease feeding shortly after ingestion. Avermectins intoxicate arthropods via contact and ingestion, although ingestion is considered to be the primary route whereby arthropods accumulate a lethal dose. The wide margin of safety for avermectin compounds to mammals is attributed to (1) the lack of glutamate-gated chloride channels in mammals; (2) the low affinity of avermectins for other mammalian ligand-gated chloride channels; and (3) their inability to readily cross the blood-brain barrier (Arena *et al*., 1995).

Potency and spectrum of activity of emamectin benzoate

Emamectin benzoate is highly potent to a broad spectrum of lepidopterous pests. LC_{90} values for emamectin benzoate against a veriety of lepidopterous pests range between 0.002–0.89 *ug*/ml (Dybas, 1989; Cox *et al.,* 1995b; Jansson and Dybas, 1996) *(Table 1)*. Emamectin hydrochloride was up to 1,500-fold more potent against armyworm species, e.g. beet armyworm, *Spodoptera exigua* (Hubner), than abamectin (Dybas *et al.,* 1989; Mrozik *et al*., 1989; Trumble *et al*., 1987). Emamectin hydrochloride was also 1,720-,884-, and 268-fold more potent to *S. eridania* than methomyl, thiodicarb, and fenvalerate, respectively, and 105- and 43-fold more toxic to cotton bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *H. virescens*, larvae than abamectin (Dybas and Babu, 1988). Recent studies showed that emamectin benzoate was 875- to 2,975-fold and 250- to 1,300- fold more potent that tebufenozide to *H. virescens* and *S. exigua*, respectively. Emamectin benzoate was also 12.5- to 20-fold and 250- to 500-fold more potent that lambda cyhalothrin and 175- to 400-fold and 2,033 to 8,600-fold more potent than fenvalerate to these two Lepidoptera, respectively (Jansson *et al*., 1997). More recent studes showed that emamectin benzoate was 1.2- to 4.8-orders of magnitude more potent to lepidopterous pests of cole crops (e.g. *S. exigua*, diamondback moth, *Plutella xylostella* (L.), and cabbage looper, *Trichoplusia ni* (Hubner) than other new insecticides, including chlorfenapyr, fipronil, and tebufenozide *(Figure 2)*.

Emamectin benzoate is markedly less toxic to most non-lepidopterous arthropods *(Table 1)*. It is about 8- to 15-fold less toxic to the serpentine leafminer, *Liriomyza trifolii* (Burgess) and the two spotted spider mite, *Tetrancychus urticae* (Koch), respectively, than abamectin (Cox *et al.,* 1995a; Dybas *et al*., 1989). Emamectin benzoate and abamectin are comparable in their potency against Mexican bean beetle, *Epilachna varivestis* Mulsant, and Colorado

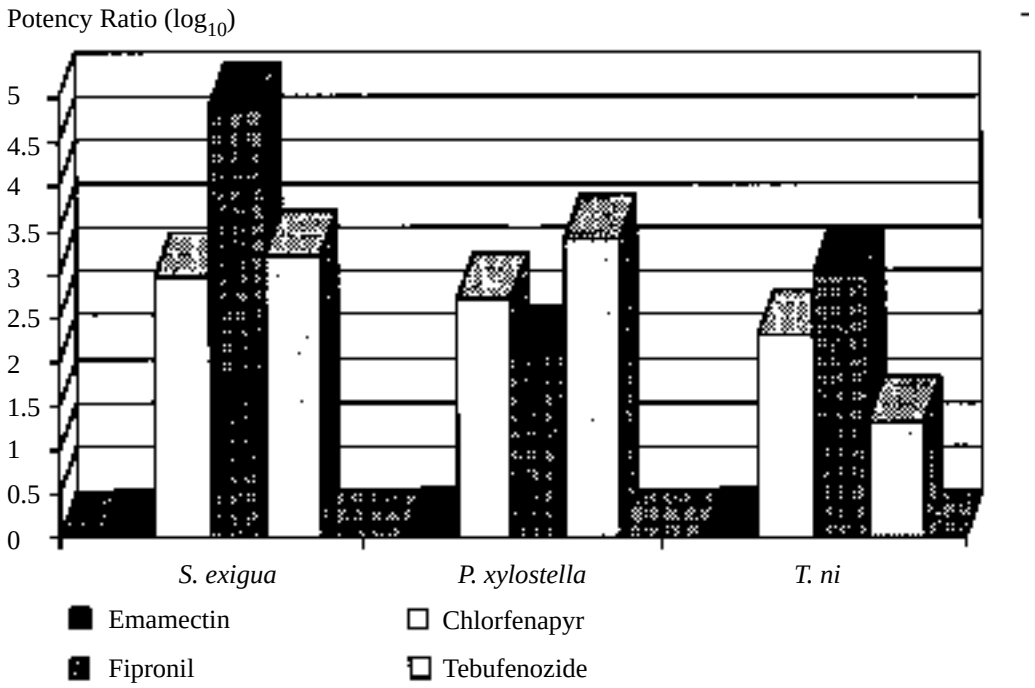


Figure 2. Log_{10}-transformed ratio of the LC_{90} value of chlorfenapyr, fipronil, or tebufenozide divided by the LC_{90} value for emamectin benzoate against three lepidopterous pests of cabbage. LC values were generated using an agar-based artificial diet assay in which different concentrations of each compound were applied to the surface of the diet (Jansson et al.*, 1997).*

Table 1. Comparative toxicity of abamectin and emamectin benzoate to different arthropod pests of agricultural importance

	LC_{90} µg/ml		
Arthropod species	Abamectin	Emamectin Benzoate	PR*
Acarina			
Foliar spray/contact assay			
Tetranychus urticae, adults	0.03[c]	0.29[d]	0.07
Insecta			
Coleoptera			
Leptinotarsa decemlineata, neonates, foliar spray	0.03[c]	0.03[d]	1.0
Epilachna varivestis, neonates, foliar spray	0.2[c]	0.2[d]	1.0
Diptera			
Liriomyza trifolii, first instar, plant dip	0.19[f]	1.45[f]	0.13
Homoptera			
Aphis fabae, foliar spray/contact	0.2–0.5[g]	19.9[d]	0.01–0.02
Lepidoptera			
Manduca sexta, neonates, foliar spray	0.02[c]	0.003[d]	7
Plutella xylostella, neonates, foliar spray	0.02[g]	0.002[g]	10
Heliothis virescens, neonates, foliar spray	0.13[d]	0.003[d]	43
Trichoplusia ni, neonates, foliar spray	1.0[c]	0.014[d]	71
Helicoverpa zea, neonates, foliar spray	1.5[c]	0.002[d]	750
Spodoptera exigua, neonates, foliar spray	1.97[g]	0.005[d]	394
Spodoptera eridania, neonates, foliar spray	6.0[c]	0.005[d]	1,200
Spodoptera frugiperda, neonates, foliar spray	25.0[c]	0.010[d]	2,500
Pseudoplusia includens, neonates, foliar spray	–	0.019[g]	–
Ostrinia nubilalis, neonates, diet assay	–	0.024[g]	–
Agrotis ipsilon, neonates, diet assay	–	0.041[g]	–
Argyrotaenia velutinana, neonates, foliar spray	–	0.009[g]	–
Cydia pomonella, neonates, diet	135.0[h]	0.89[h]	152

[a]PR, potency ratio = LC_{90} abamectin/LC_{90} emamectin benzoate
[b]Royalty and Perring (1987)
[c]Dybas and Green (1984)
[d]Dybas *et al.* (1989)
[e]Dybas (1989)
[f]Cox *et al.* (1995a)
[g]Merck, unpublished data
[h]Cox *et al.* (1995b)

potato beetle, *Leptinostarsa decemlineata* (Say) (Dybas, 1989). Emamectin benzoate is markedly less toxic to black bean aphid, *Aphis fabae* Scopoli, than abamectin *(Table 1)*.

Like abamectin, emamectin benzoate is less toxic to most beneficial arthropods (e.g., honey bees, parasitoids, predators), especially when exposure occurs beyond one day after application (Lasota and Dybas, 1991 and references therein; Cox *et a*l., unpublished). Foliar residues of emamectin benzoate were only slightly toxic (<20% mortality) to most beneficial insects, including honey bees, *Apis mellifera*, and several predtors and parasitoids, within one day after application and often within a few hours after application (Cox *et al*., unpublished). The low toxicity was related to the short half-life of emamectin benzoate on foliage. On celery, the half-life of foliar dislodgeable residues was estimated to be approximately 0.66 days (Dunbar *et al.*, unpublished). Kok *et al*. (1996) showed that emamectin hydrochloride (MK-0234) displayed minimal adverse effects against two hymenopterous parasitoids (*Pteromalus puparum* and *Cotesia orobenae*) on broccoli. Like abamectin, emamectin benzoate provides ecological selectivity (and in some cases physiological selectivity) to a wide range of beneficial arthropods. For this reason, it is compatible with integrated pest management (IPM) programs.

Photostability and translaminar movement

Abamectin and emamectin benzoate are very susceptible to photodegradation. MacConnell *et al.* (1989) showed that the half-life of abamectin was <10 h in simulated sunlight and that there were marked differences in the half-life of abamectin on Petri dishes and on leaves in light and dark environments. The half-life of emamectin benzoate on celery has been estimated to be 0.66 days; on cole crops, the half-life is expected to be even shorter. Numerous photodegradates of emamectin benzoate have been identified (Feely *et al*., 1992).

Despite the short half-life for avermectin insecticides in sunlight, low levels of these compounds are taken up rapidly via translaminar movement into foliage. Translaminar movement of abamectin has been demonstrated in numerous studies (Dybas, 1989 and references therein: Wright *et al*., 1985). Presence of abamectin and emamectin benzoate reservoirs in parenchyma tissue accounts for their long residual activity on certain crops under field conditions, and their ability to control several Dipteran and Lepidopteran leafminers (Jansson & Dybas 1996).

Field efficacy

Excellent efficacy of this compound at low use rates (7.5–16.8 g ai/ha) has been demonstrated against numerous lepidopterous pests in a variety of crops (Jansson and Lecrone, 1991; Jansson *et al*.,1996; Leibee *et al.*, 1995; Merck, unpublished). Results from numerous field trials conducted in cruciferous crops were very consistent. In 29 trials conducted to compare efficacy of emamectin benzoate (8.4, 11.2, and 16.8 g ai/ha) with methomyl (504 g ai/ha), permethrin (112 g ai/ha), and methomyl (504 g ai/ha) in combination with permethrin (112 g ai/ha), emamectin benzoate was superior to the other insecticides at controlling all lepidopterous pests (*P. xylostella, T. ni, S. exigua*) on cole crops and resulted in higher percentages of marketable heads than all other insecticides tested *(Figure 3)*. All three rates of emamectin benzoate were equal in their effectiveness at controlling these pests. Similar results were found when only data from trials that included *P. xylostella* were included (16 trials) *(Figure 4)*. In 21 other field trials conducted to compare the effectiveness of emamectin benzoate (8.4 g ai/ha) with biological insecticides, *Bacillus thuringensis* (Berliner) ssp. *aizawai* (XenTari, 1,120 g ai product/ha), *B. thuringiensis* ssp. *kurstaki* (Dipel, 1,120 g ai product/ha), and treatment regimes that rotated two consecutive weekly applications of emamectin benzoate with two consecutive weekly applications of XenTari, emamectin benzoate was superior to *B. thuringiensis* ssp. *aizawai (Figure 5)*. Similar results were found in Florida (Leibee et al., 1995). Collectively, these data demonstrate that emamectin benzoate produces superior efficacy at controlling a broad spectrum of lepidopterous pests and increases in yield of cole crops compared with other commonly used chemical and biological insecticides.

Cross resistance and resistance management

Cross resistance between abamectin and amamectin benzoate and other classes of chemistry has not been documented widely, nor is it well understood. Recent studies by Lasota *et al.* (1996) showed that there was no cross resistance between abamectin, emamectin benzoate, permethrin and methomyl in *P. xylostella* using an ingestion bioassay. Additional evidence from studies conducted at Merck Research Laboratories also suggested that there was no cross resistance between abamectin and emamectin benzoate in *T. urticae* and *L. trifolii* (Jansson *et al.*, unpublished); however, more work is needed to confirm this belief.

Pro-active resistance management programs were developed for abamectin. Similar programs are being formulated for emamectin benzoate. Part of this pro-active strategy includes the development of monitoring systems to detect resistance in high risk populations of arthropods. Baseline data for susceptibility of *P. xylostella* populations to emamectin benzoate have been generated (Lasota *et al.*, 1996). Monitoring programs for *P. xylostella* and other problematic Lepidoptera are planned.

To minimize the risk of resistance to emamectin benzoate in lepidopterous pests, most product labels will provide restrictions on the type, number, and sequencing of applications allowed per growing season. In addition, applications should be excluded from transplant nurseries of certain vegetable crops to reduce selection pressure on arthropods that pose the greatest risk (e.g. *P. xylostella*). These and other

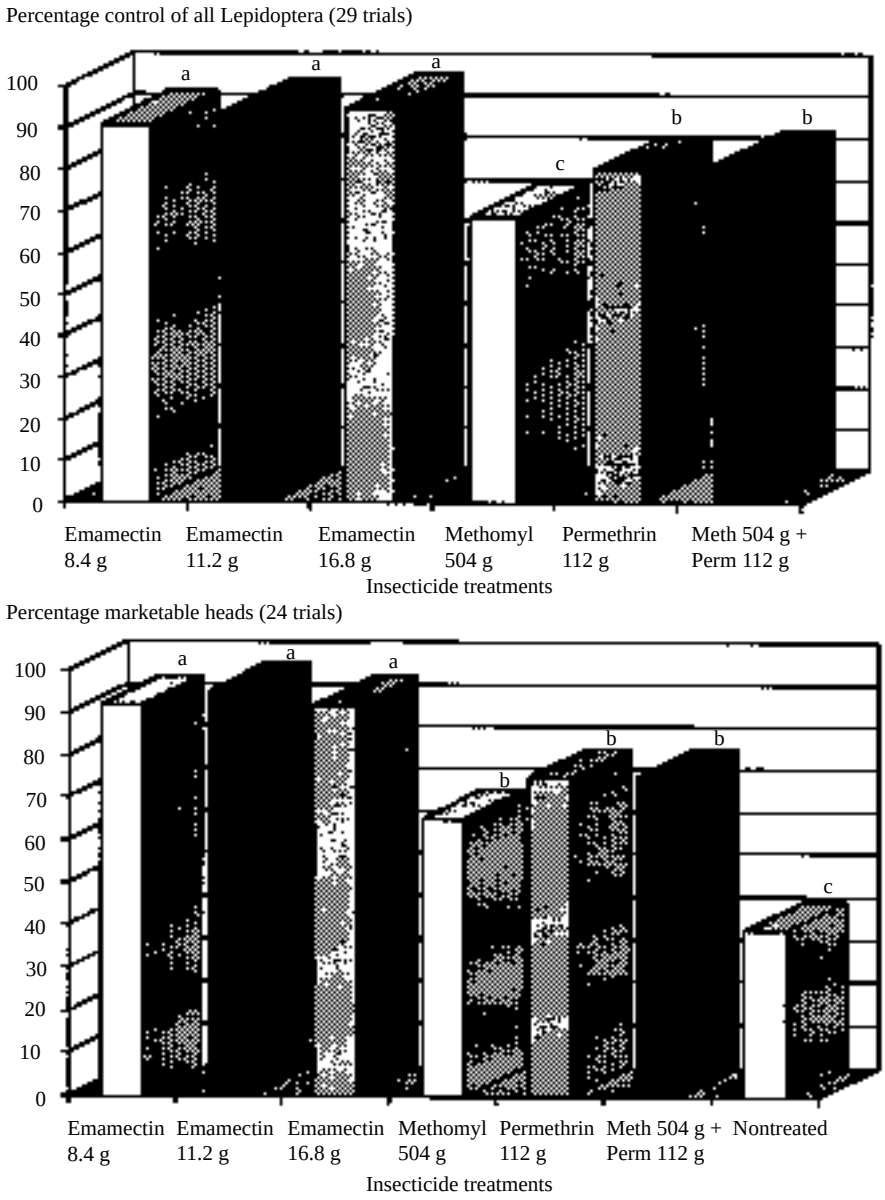


Figure 3. Percentage control of all lepidopterous pests (compared with nontreated plants) (top) and percentage marketable heads (bottom) per treatment in cabbage plots treat with different insecticides (emamectin benzoate, 8.4, 11.2, and 16.8 g ai/ha; methomyl, 504 g ai/ha; permethrin 112 g ai/ha; methomyl, 504 g ai/ha, in combination with permethrin, 112 g ai/ha). Data are from 29 (top) and 24 field trials (bottom).

strategies, such as advocation of rotation of emamectin benzoate with ohter chemical and biological insecticides with different modes of action, and advocation of IPM programs in all crops, should help to prolong the life of emamectin benzoate in the commercial sector.

References

Arena, J. P. (1994). Expression of *Caenorhabditis elegans* mRNA in *Xenophus* oocytes: a model system to study the mechanism of action of avermectins. *Parasitology Today* ***10:*** 35–37

Arena, J. P., Liu, K. K., Paress, P. S., Frazier, E. G., Cully, D. F., Mrozik, H. and Schaeffer, J. (1995). The mechanism of action of avermectin in *Caenorhabditis elegans*: correlation between activation of glutamate-sensitive chloride current, membrane binding and biological activity. *Journal of Parasitology* ***81:*** 286–294

Campbell, W. C. (ed.) (1989). Ivermectin and abamectin. Springer, New York, 363 pp.

Cox, D. L., Remick, D., Lasota, J.A. and Dybas, R. A. (1995a). Toxicity of avermectins to *Liriomyza trifolii* (Diptera: Agromyzidae) larvae and adults. *Journal of Economic Entomology* ***88:*** 1415–1419

Cox, D. L., Knight, A. L. , Biddinger, D. G., Lasota, J. A., Pikounis, B., Hull, L. A. and Dybas, R. A. (1995b). Toxicity and field efficacy of avermectins against codling moth (Lepidoptera: Tortricidae) on apples. *Journal of Economic Entomology* ***88:*** 708–715

Cvetovich, R. J. Kelly, D. H., Di Michele, L. M., Shuman, R.F. and Grabowski, E. J. J. (1994). Synthesis of 4"-epi-amino-4"-deoxyavermectins B_1. *Journal of Organic Chemistry* ***59:*** 7704–7708

Dybas, R. A. (1989). Abamectin use in crop protection. *In: Ivermectin* and *Abamectin* (ed. W.C. Campbell) pp. 287–310. Springer, New York

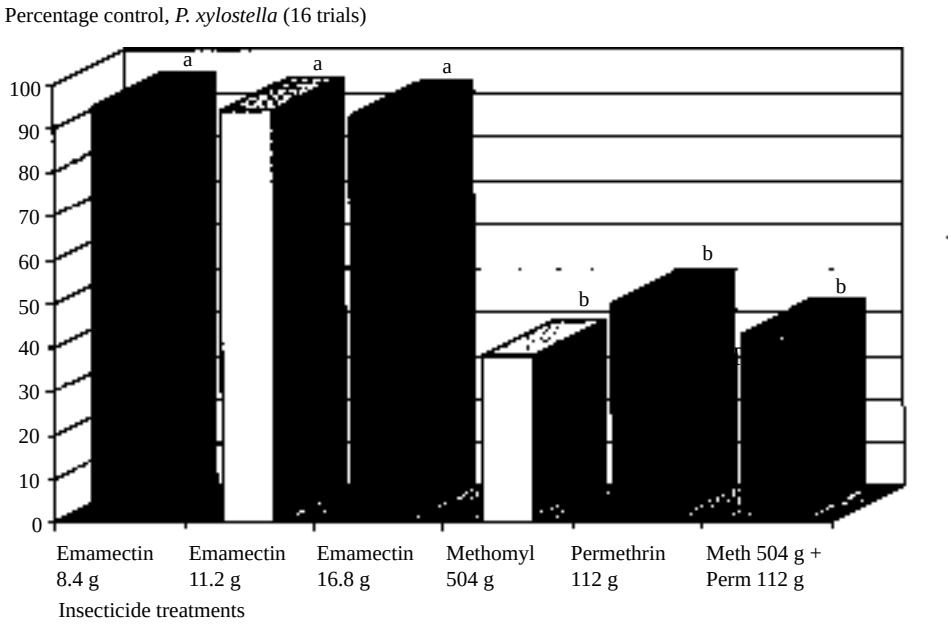


Figure 4. Percentage control of P. xylostella *populations (compared with nontreated plants) in cabbage plots treated with different insecticides (see* Fig. 3 *details). Data are from 16 field trials.*

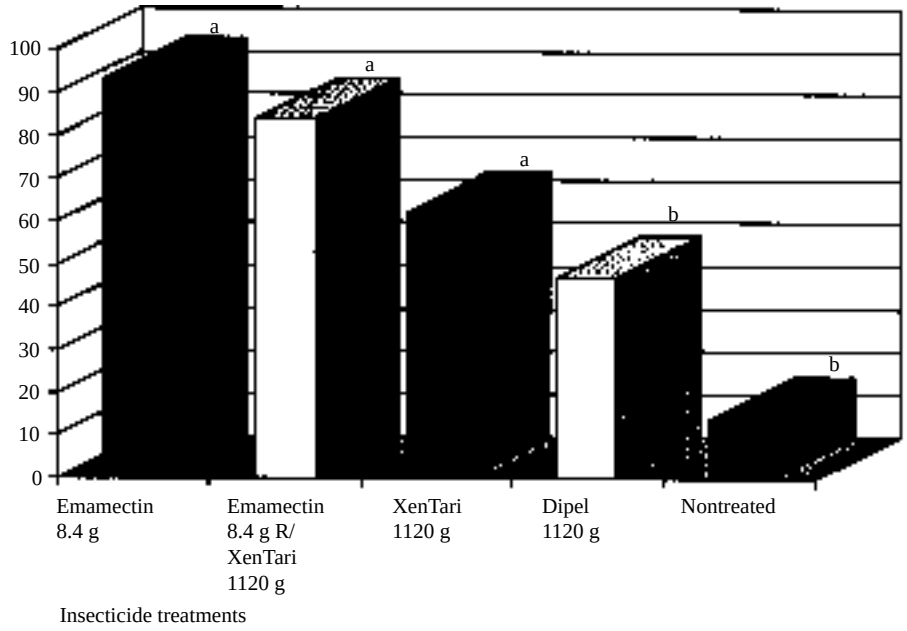


Figure 5. Percentage marketable heads of cabbage plants treated with emamectin benzoate (8.4 g ai/ha), emamectin benzoate (8.4 g ai/ha) rotated at 2–3 weeks intervals with B. thuringiensis ssp. aizawai (XenTari, 1,120 g product/ha), B. thuringiensis *ssp.* aizawai *(XenTari, 1,120 g product/ha), or* B. thuringiensis *ssp.* kurstaki *(Dipel, 1,120 g product/ha), and nontreated plants. Data are from 21 field trials.*

Dybas, R. A. and Babu, J. R. (1988): a novel avermectin insecticide for crop protection. *In:* British Crop Protection Conference. Pests and Diseases pp. 57–64. British Crop Protection Council, Croydon, U.K.

Dybas, R. A. and A. S. J. Green. (1984). Avermectins: their chemistry and pesticidal activity. *In:* British Crop Protection Conference. Pests and Diseases pp. 947–954. Birtish Crop Protection Council, Croydon, U.K.

Dybas, R. A. Hilton, N. J., Babu, J. R., Preiser, F. A. and Dolce, G. J. (1989). Novel second-generation avermectin insecticides and miticides for crop protection. *In: Novel Microbial Products for Medicine and Agriculture* (ed. A.L. Demain *et al.*) pp. 203–212. Society Industrial Microbiology, Annandale, VA.

Feely, W. F., Crouch, L. S., Arison, B. H., Vanden Heuvel, W. J. A., Colwell, L. F. and Wislocki, P. G. (1992). Photodegradation of 4"-(epimethylamino)-4"-deoxyavermectin B1a thin films on glass. *Journal of Agricultural and Food Chemistry* ***40:*** 691–696

Fisher, M. H. and Mrozik, H. (1984). The avermectin family of macrolide-like antibiotics. *In: Macrolide Antibiotics* (ed. S. Omura) pp. 553–606. Academic Press, New York

Jansson, R. K. and Dybas, R. A. (1996). Avermectins, biochemical mode of action, biological activity, and agricultural importance. In: *Insecticides with Novel Modes of Action: Mechanism and Application* (ed. I. Ishaaya) Springer, New York (in press).

Jansson, R. K. and Lecrone, S. H. (1991). Efficacy of nonconventional insecticides for control of diamondback moth, *Plutella xylostella* (L.), in 1991. *Proceedings of the Florida State Horticultural Society* ***104:*** 279–284

Jansson, R. K., Halliday, W. R., Argentine, J. A. and Dybas, R. A. (1997). Comparison of miniature and high volume bioassays for screening insecticides. *Journal of Economic Entomology* (in review)

Jansson, R. K., Peterson, R. F., Mookerjee, P. K., Halliday, W. R. and Dybas, R. A. (1996). Efficacy of solid formulations of emamectin benzoate at controlling lepidopterous pests. *Florida Entomologist* ***79:*** 434–449

Kok, L. T., Lasota, J. A., McAvoy, T. J. and Dybas, R. A. (1996). Residual foliar toxicity of 4"-epimethylamino-4"-deoxyavermectin B1 hydrochloride (MK-243) and selected commercial insecticides to adult hymenopterous parasites, *Pteromalus puparum* (Hymenoptera: Pteromalidae) and *Cotesia orobenae* (Hymenoptera: Braconidae). *Journal of Economic Entomology* ***89:*** 63–67

Lasota, J. A. and Dybas, R. A. (1991). Avermectins, a novel class of compounds: implications for use in arthropod pest control. *Annual Review of Entomology* ***36:*** 91–117

Lasota, J. A., Shelton, A. M., Bolognese, J. A. and Dybas, R. A. (1996). Baseline toxicity of avermectins to diamondback moth (Lepidoptera: Plutellidae) populations: implications for susceptibility monitoring. *Journal of Economic Entomology* ***89:*** 33–38

Larivieri, M., Aziz, M., Weimann, D., Ginoux, J., Gaxotte, P., Vingtain, P., Beauvais, B., Derouin, F., Schulz-Key, H., Basset, D. and Sarfati, C. (1985). Double-blind study of ivermectin and diethylcarbamazine in African onchocerciasis patients with ocular involvement. *Lancet* ***2:*** 174–177

Leibee, G. L., Jansson, R. K., Nuessly, G. and Taylor, J. (1995). Efficacy of emamectin benzoate and *Bacillus thuringiensis* for control of diamondback moth on cabbage in Florida. *Florida Entomologist* ***78:*** 82–96

MacConnell, J. G., Demchak, R. J., Preiser, F. A. and Dybas, R. A. (1989). Relative stability, toxicity, and penetrability of abamectin and its 8,9-oxide. *Journal of Agricultural and Food Chemistry* ***37:*** 1498–1501

Mrozik, H. (1994). Advances in research and development of avermectins. *In: Natural and Engineered Pest Management Agents*, American Chemical Society Symposium Series No. 551. (ed. P. A. Hedin *et al.*) pp. 54–73. American Chemical Society, Washington

Mrozik, H., Eskola, P., Linn, B. O., Lusi, A., Shih, T. L., Tishler, M., Waksmunski, F. S., Wyvratt, M. J., Hilton, N. J., Andersen, T. E., Babu, J. R., Dybas, R. R., Preiser, F. A. and Fisher, M. H. (1989). Discovery of novel avermectins with unprecedented insecticidal activity. *Experientia* ***45:*** 315–316

Rohrer, S. P. and Arena, J. P. (1995). Ivermectin interactions with invertebrate ion channels. *In: Molecular Action of Insecticides on Ion Channels. American Chemical Society Symposium series* No. 591 (ed. J.M. Clark) pp. 264–283. American Chemical Society, Washington, D.C.

Royalty, R. N. and Perring, T. M. (1987). Comparative toxicity of acaricides to *Aculops lycopersici and Homeopronematurs anconai* (Acari: Eirophydae, Tydeidae). *Journal of Economic Entomology* ***80:*** 348–351

Trumble, J. T., Moar, W. J., Babu, J. R. and Dybas, R. A. (1987). Laboratory bioassays of the acute and antifeedant effects of avermectin B1 and a related analogue on *Spodoptera exigua* (Hubner). *Journal of Agricultural Entomology* ***4:*** 21–28

Turner, M. J. and Schaeffer, J. M. (1989). Mode of action of ivermectin. *In: Ivermectin and Abamectin* (ed. W.C. Cambell) pp. 73–88. Springer, New York

Wright, D. J., Loy, A., Green, A. S. J. and Dybas, R.A. (1985). The translaminar activity of abamectin (MK-936) against mites and aphids. *Med Fac Landbouww Rijksuniv Gent* ***50(2b):*** 633–637

Management of diamondback moth with emamectin benzoate and *Bacillus thuringiensis* subsp. *aizawai* insecticides

Ronald F. L. Mau[1], Dennis M. Dunbar[2], Laura Gusukuma-Minuto[1] and Robin S. Shimabuku[3]

[1]*Department of Entomology, College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, Honolulu, Hawaii 96822*

[2]*Merck Research Laboratories, 7555 North Del Mar Avenue, Suite 204, Fresno, California 93701*

[3]*Hawaii Cooperative Extension Service, 310 Kaahumanu Avenue, Building 214, Kahului, Hawaii 96732*

Abstract

Emamectin benzoate (= MK-0244) was evaluated with *Bacillus thuringiensis kurstaki* (Btk), *B.t. aizawai* (Bta) and other insecticides against diamondback moth, *Plutella xylostella* (L.), (DBM) on cabbage in experimental field plots and on grower farms. Conventional insecticides were ineffective against DBM and resulted in poor yields. Bta insecticides provided moderate levels of DBM control and low to moderate cabbage yields. Emamectin benzoate provided superior control of DBM and other lepidopteran larvae in all on-station experiments as well as in field trials on four commercial farms. Growers obtained an average increase in marketable yield of 29 percent and an estimated net increase in gross revenues of about $660 per hectare.

Commercial use of emamectin benzoate is being developed for use on vegetable crops in the U.S. under the trademark PROCLAIM® 5 SG. This insecticide has positive characteristics for use in IPM programs. Its rapid degradation on leaf surfaces provides a good margin of safety for parasitoids of DBM and other pest species. Translaminar residual activity provides excellent protection against larvae of DBM and other cruciferous Lepidopterans.

Key words: IPM, cabbage, biorational insecticides

Introduction

The major cabbage crops grown in Hawaii are green head and Chinese (Napa) cabbage. About 405 hectares of both types of cabbage are produced annually primarily for local consumption. Cabbage is cropped throughout the year in sequential plots. The majority of growers plant and harvest cabbage on weekly schedules.

The major lepidopteran pests in order of importance are DBM, imported cabbage webworm *(Hellula undalis)*, imported cabbageworm *(Pieris rapae)* and cabbage looper *(Trichoplusia ni)*. DBM is the key Lepidopteran pest of cabbage in Hawaii. It occurs on all islands where crucifers are grown. DBM causes extensive losses primarily during the period April through October, but it has been a problem during other times of the year. DBM is a formidable pest due its prolific nature and ineffectiveness of registered pesticides (Tabashnik *et al.* 1990, 1992; Mau *et al.* 1995a, 1995b, 1995c, 1995d). Although selection of tolerant cabbage varieties and conservation of parasitoids are viable management tactics, it has been very difficult to provide economic control of the pest without an effective insecticide.

During the past five years, the cabbage industry has lost nearly $1.3 million due to DBM related causes. Economic losses occur as a result of direct feeding damage to the cabbage, physical presence of caterpillars within marketed heads, additional harvest labor costs, and increased insecticide and application expenses. A case study was conducted for the island of Maui. DBM reduced marketable cabbage yields practically every year during the past 9 years. The overall yield reduction during the period was 40 percent, and this occurred despite a 15 percent increase in harvested acres.

Yields have decreased because of the lack of an effective insecticide that can be used when treatment threshold is surpassed. Bta products are somewhat effective during periods of lower DBM population density, but they do not provide adequate protection at higher population densities. Other non-chemical tactics provide supplementary protection and have not provided adequate levels of control on commercial farms.

The results from laboratory and field evaluations of a second generation avermectin insecticide, emamectin benzoate (= MK-0244) and two Bta products are presented in this paper. Emamectin benzoate is a novel semi-synthetic avermectin insecticide derived from the fermentation product, avermectin B_1 (abamectin). It is a second generation avermectin insecticide that is a chemically modified derivative of abamectin. Other closely related compounds were found to be extremely active on lepidopteran larvae (Dybas and Babu, 1988; Dybas *et al.*, 1989; Lasota and Dybas, 1991).

Materials and Methods

Bioassay tests Laboratory bioassays were performed using larvae obtained from a laboratory colony of DBM established from field collections of DBM at Kamuela, Hawaii. The larvae were reared on head cabbage leaves. Head cabbage variety 'C-G Hybrid' was seeded in community pots. Each pot contained approximately 10 cabbage plants. The potted seedlings were at the 5–7 true leaf stage when they were treated.

One liter of each insecticide solution was prepared by diluting the appropriate amount of insecticide with water. The spray adjuvant Nu-film P (Miller Chemical & Fertilizer Corporation) was added at a rate of 0.47 ml per l. Treatment was accomplished by inverting the community pot and completely dipping the plants in the appropriate insecticide solution. The plants were allowed to air dry before they were used.

The bioassays were performed at 75 °C, 76 % RH and a 24 hour photoperiod. After placement on the leaves, mortality was recorded daily. The original leaves were replaced with new leaves from the corresponding treated plants two days later.

Larvae were reported as dead when there was no movement of the larva when probed. Results are reported as percent mortality. Actual mortality was transformed using arcsine transformation and the data set was subjected to analysis of variance (SAS Institute, version 6.04). Means were separated using Tukey's studentized range test.

Bioassays with neonate DBM larvae The efficacy of emamectin benzoate 0.16EC (3.4 g a.i.per 378.54 l) was evaluated against four *Bacillus thuringiensis* (Bt) insecticides. The Bt products were Dipel 2X (Abbott Laboratories), Mattch Bio-insecticide (Mycogen Corp.), MVP (Mycogen Corp.) and Xentari (Abbott Laboratories). Ten neonate DBM larvae were placed on each treated leaf. All treatments were replicated 10 times.

Bioassays with fourth instar DBM larvae Feeding studies were conducted on cabbage treated with emamectin benzoate 0.16EC at 3.4 g a.i. per 378.5 liters of water. Ten fourth instar larvae placed on leaves treated 24 hr earlier. Mortality observations were made 12 and 24 hours after placement.

Field studies Three season-long field experiments were conducted during 1995 against DBM, imported cabbage webworm *(Hellula undalis)*, imported cabbageworm *(Pieris rapae)*, and cabbage looper *(Trichoplusia ni)*. The experiment station trials were conducted during March to November at the University of Hawaii, College of Tropical Agriculture and Human Resources experimental farm at the Maui Agricultural Park. The farm is located at the 1,400 ft. elevation of Mt. Haleakala on the Hawaiian island of Maui.

Each of the field trials utilized randomized complete block designs with four replications (blocks). Each treatment plot measured 0.0039 ha. Treatment plots in each block were separated by a 1.67 m. row spacing. Blocks were separated by a 3 m. wide strip that allowed sprayer access. Total field size was 0.118 ha. Mean daily temperatures during the three studies was 22, 23.6, and 22.9 °C, respectively. The mean daily rainfall was 8.9, 0.5, and 2.3 mm, respectively.

Head cabbage variety 'Tastie' (Takii Seed Co.) was used in all tests. Cabbage seedlings in the 3–5 true leaf stage were planted into each plot in 4 rows on 0.91 m. centers and 0.457 m. within row spacing. Adjacent rows were offset to allow equidistant plant spacing between rows. The field was irrigated by overhead sprinklers at 2–3 day intervals. Irrigation was withheld for at least 24 hours after each spray application.

Treatments were made at 7-day intervals until one week before harvest. The non-ionic surfactant, Excel 90 (Brewer Environmental Industries, Inc.) was added at a rate of 585 ml per ha. A total of 7 applications were made during each field test. Each treatment was applied at a pressure of 3.2 kg per cm^2 and a rate of 1,169 l per ha using a PTO driven tractor mounted sprayer. The spray boom was fitted with one hollow cone TX-18 sprayer nozzles (Spraying Systems Co., Wheaton, Illinois) placed directly over each row.

Harvest evaluations were performed 8 weeks after transplanting. Data collected for each plant were Lepidopteran numbers, damage ranks, and marketability. The following stratified sampling method was used to select the plants for evaluation. The remaining plants in the two center rows were numbered from 1–25. The second row was numbered from the opposite end of the plot. Every fifth plant was harvested until a total of 10 heads had been harvested. The loose wrapper leaves were removed from each head before data was collected.

Larval feeding damage ranks (0–5) were assigned for each head. A '0' rank was assigned if there were no damage. The numerical rating increased as the degree of caterpillar damage increased. A '5' rating was assigned where there was extensive larval damage. A marketability assessment was made after each head was ranked for damage and dissected for pest numbers. The marketability assessments followed procedures used by commercial growers and was based on the severity of insect damage on the wrapper and inner leaves of the head.

Lepidopteran pest data were determined by removing and inspecting ten randomly selected plants. A pre-treatment census for lepidopteran pests was performed the day before the first treatment. Subsequent pest evaluations were subsequently performed at two week intervals. This was done six days after prior treatment.

Commercial farm tests Four commercial growers were selected for commercial validation tests under a U.S. EPA approved experimental use permit (EUP) for PROCLAIM® 0.16 EC Insecticide. Two growers

were located in the Kula district on the island of Maui and the remaining two growers were located in the Kamuela district on the island of Hawaii. All growers applied PROCLAIM® 0.16 EC insecticide at a rate of 3.4 g a.i. per ha. Treatments began immediately after the cabbage was transplanted and were repeated at 7–8-day intervals. The final application was made 7-days before harvest. Data on lepidopteran larvae numbers were taken immediately prior to the first treatment, 6 days after the third, 6 days after the fifth treatment.

Comparisons of the performance of PROCLAIM® 0.16 EC treatments were made against each grower's standard pest control practice. Their standard practices varied considerably.

Grower 1 used a combination of Xentari at 367 g/ha and naled 8 E at 383 ml/ha a total of 10 times at 3–7-day intervals.

Grower 2 made a total of 13 treatments to the grower standard field. He applied Xentari at 91.7 g/ha and mevinphos at 574 ml/ha was applied at 7-day intervals for the first two treatments. Three days later an application on MVP at 766 ml/ha was made. This was followed by an application of Xentari at 91.7 g/ha and methomyl at 183.7 g/ha four days later. Dipel 2X at 183.7 g/ha and mevinphos at 574 ml/ha was applied three days later followed by Xentari at 137.6 g/ha three days later. Thereafter, the following applications were consecutively made at 3–4 day intervals: MVP at 766 ml/ha, Xentari at 137.6 g/ha, Dipel 2X at 183.7 g/ha, MVP at 766 ml/ha, Xentari at 137.6 g/ha, Dipel 2X at 183.7 g/ha, and MVP at 766 ml/ha.

Grower 3 made 5 applications of Xentari at 183.7 g/ha at 7-day intervals. No other insecticide applications were made.

Grower 4 sprayed to control DBM adults as well as larvae. He first treated with a combination of mevinphos at 355 ml/ha and Xentari at 183.7 g/ha. It was followed by an application of Xentari at 183.7 g/ha three days later. Subsequently, the following consecutive treatments at 7-days intervals were naled 191 ml/ha; Xentari at 183.7 g/ha; Ambush at 207 ml/ha; mevinphos at 355 ml/ha; Xentari at 183.7 g/ha.

Harvest evaluations were made for the emamectin benzoate treatment and from an adjacent plot that were treated with the growers' standard pest management protocol. Ten plants were randomly selected from each of the plots and harvested using commercial protocols. Data was collected in the same manner described above for the experiment station tests.

Statistical analysis The number of immatures counted were subjected to analysis of variance (ANOVA). Mean separation was accomplished using Tukey's mean separation test (SAS for Windows version 6.08).

Results

Laboratory DBM Feeding Studies

Bioassays with neonate DBM larvae Leaf ingestion bioassays were conducted to compare the relative effectiveness of emamectin benzoate, Btk, and Bta products against neonate DBM larvae. The emamectin benzoate (= MK-0244) treatment gave very rapid kill compared with that of the Btk and Bta products *(Table 1)*. All of the DBM larvae died within 1 day after placement on treated leaves. In comparison, none of the Btk (Dipel 2X and MVP) and Bta (Mattch and Xentari) treatments gave complete kill, and mortality in all of the Bt treatments increased more slowly than in the emamectin benzoate treatment. Bta treatments were more effective than Btk treatments. Mattch gave the best overall control among all of the Bt treatments.

Bioassays with fourth instar DBM larvae Larvae fed briefly and most of them ceased feeding within 4–8 hours after placement. Fifty one percent of the larvae were paralyzed within 4 hours after placement. Seventy-one percent of the larvae were paralyzed within 8 hours of placement, and all of the larvae were paralyzed within 24 hours after placement. All larvae died within 48 hours after placement.

Comparative studies showed that the total leaf area consumed by a fourth instar larva in the

Table 1. Evaluation of emamectin benzoate (MK-0244, 0.16 EC) and *Bacillus thuringiensis* insecticides against diamondback moth larvae

Treatment rate per ha		Mean percent mortality (DAT)			
		1	2	3	4
Mattch	4.66 l	5.2b	42.3b	70.2b	83.9abc
Mattch	2.33 l	3.2b	36.8b	71.8b	82.1ab
Xentari	1.12 kg	9.2b	22.9bc	46.9bc	61.7bcd
Xentari	0.56 kg	9.4b	37.2b	49.1bc	57.7bcd
Dipel 2X	2.24 kg	11.0b	15.9bc	28.5cd	51.9cd
MVP	4.66 l	0.0b	15.3bc	34.8cd	49.7d
MK-0244 0.16 EC	72 ml	100.0a	100.0a	100.0a	100.0a
Untreated check	–	4.7b	7.7c	9.7d	11.5e

DAT = days after treatment. Numbers within the same column followed by a different letter are significantly different (P<0.0001, Tukey's studentized test, SAS Version 6.04). Data were subjected to arcsine transformation prior to analysis. Untransformed mean percent mortality is presented. Nu-Film P @ 0.47 ml/ l of spray solution was used as a surfactant to assist in wetting the cabbage leaves.

emamectin benzoate treatment was slightly larger than its head capsule (0.55 cm^2). Larvae on untreated leaves consumed about ten times more tissue (5.7 cm^2).

The residual activity of emamectin benzoate within treated leaves was estimated using 3rd and 4th instar DBM larvae. Placements were made on leaves excised from treated plants 0, 1, 2, 3, 7, and 10 days after treatment. The plants were held in an enclosed greenhouse after treatment. Emamectin benzoate residues were highly active for the entire duration of the experiment. Ninety-four to one hundred percent of the larvae died within 3 days of placement in all of the post-treatment residue groups. These results demonstrated that emamectin benzoate could conceivably provide adequate levels of DBM control in the field for 10 days.

On-station experiments

Both of the field experiments were conducted during periods when DBM populations were high. Both formulations of emamectin benzoate provided excellent control of DBM and kept larval densities at relatively low levels when compared with other treatments *(Figures 1* and *2)*. *Bacillus thuringiensis* treatments (Xentari, Dipel 2X, and Mattch) provided only moderate levels of control when compared to the Untreated Check (UTC) and emamectin benzoate treatments. Permethrin, methomyl, and endosulfan did not provide any DBM control.

In these and other field experiments, we observed increased larval densities about 2–3 weeks after transplanting. It appears that this is due to a combination spray coverage and efficiency of the treatment issues. It is difficult to effectively deliver sprays to the apical bud because of the cupped leaves.

There were very distinct differences in marketable yield among the treatments. Both formulations of emamectin benzoate provided the best overall yield (88–95%) in both experiments. Yield from *Bacillus thuringiensis* treatments varied from poor to moderate (18–68% yield). Mattch provided moderate yields when used at the 0.77 l per ha application rate. There was no marketable yield from the permethrin, methomyl, endosulfan and the Untreated Check treatments.

Grower evaluation of PROCLAIM® 0.16 EC insecticide

Significantly better control of DBM was obtained with fewer pesticide applications of PROCLAIM® 0.16 EC compared with their respective standard programs. The harvest DBM densities presented in *Figure 3* are indicative of DBM larval densities that were maintained through the crop season. PROCLAIM® 0.16 EC provided rapid kill of DBM larvae and consequently very little direct damage occurred in these plots. DBM larvae were scarce in PROCLAIM® 0.16 EC treated plots compared with that of the growers standard program.

There were significant increases in marketable cabbage yields in fields treated with PROCLAIM® 0.16 EC compared with those with each grower's standard program *(Figure 4)*. Yields of all PROCLAIM® 0.16 EC treated fields exceeded the comparison fields and the Cooperative Extension average yield target (613 kg/ha). In two cases, PROCLAIM® 0.16 EC yields exceeded Extension's maximum yield target (7343 kg/ha).

Yields in three of four tests using standard grower practices fell short of extension's estimate for average

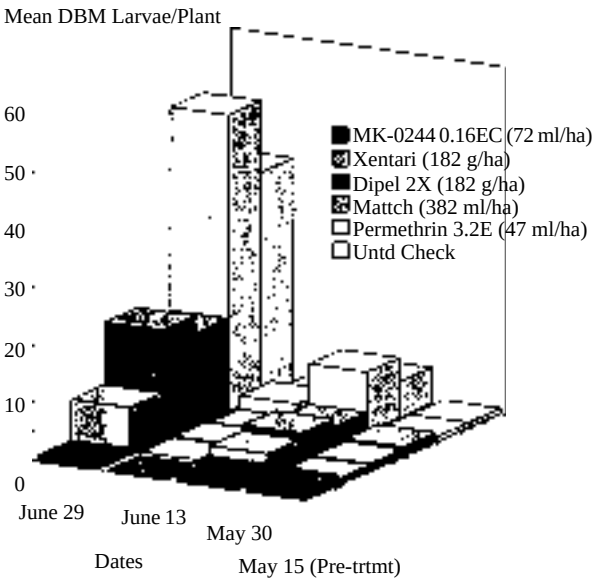


Figure 1a. Evaluation of emamectin benzoate 0.16 EC and other insecticides against diamondback moth larvae. Comparison of larval number at different stages of crop growth.

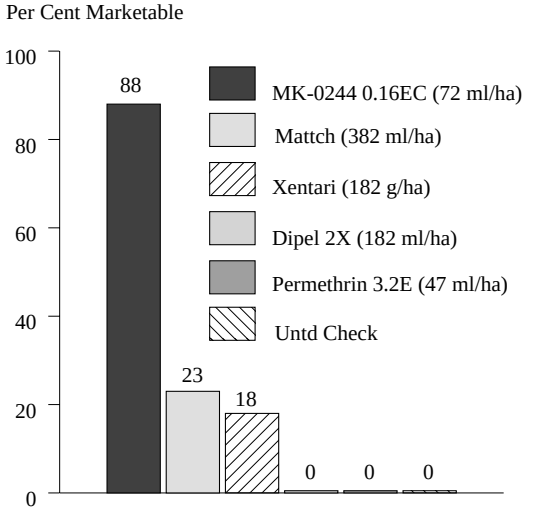


Figure 1b. Evaluation of emamectin benzoate 0.16 EC and other insecticides against diamondback moth larvae. Comparison of harvest yields.

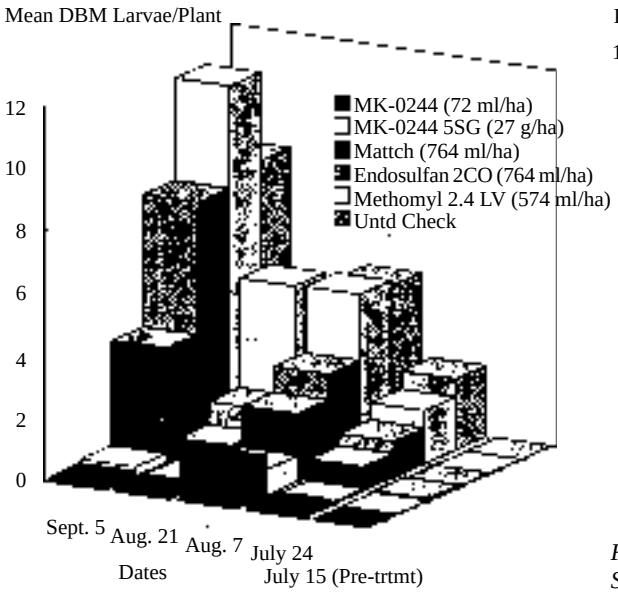


Figure 2a. Evaluation of emamectin benzoate 0.16 EC, 5 SG and other insecticides against diamondback moth larvae. Comparison of larval numbers at different stages of crop growth.

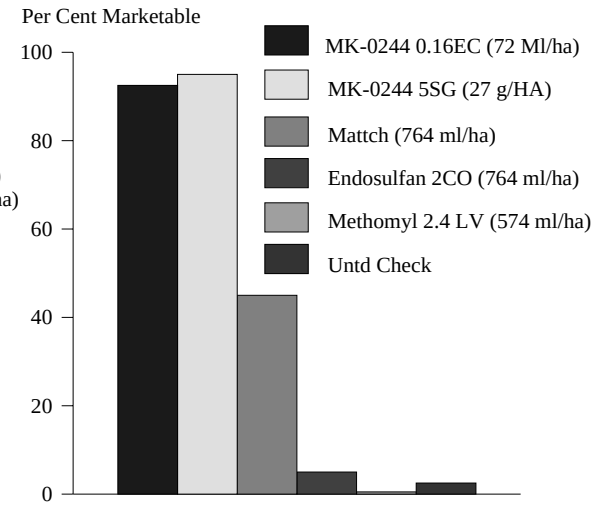


Figure 2b. Evaluation of emamectin benzoate 0.16 EC, 5 SG and other insecticides against diamondback moth larvae. Comparison of larval numbers at different stages of crop growth.

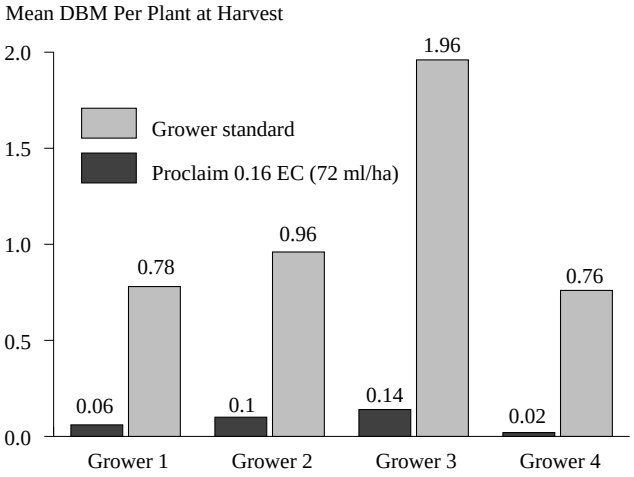


Figure 3a. Evaluation of PROCLAIM 0.16 EC by commercial growers in Hawaii. Comparison of DBM larval control with PROCLAIM and the growers standard treatment.

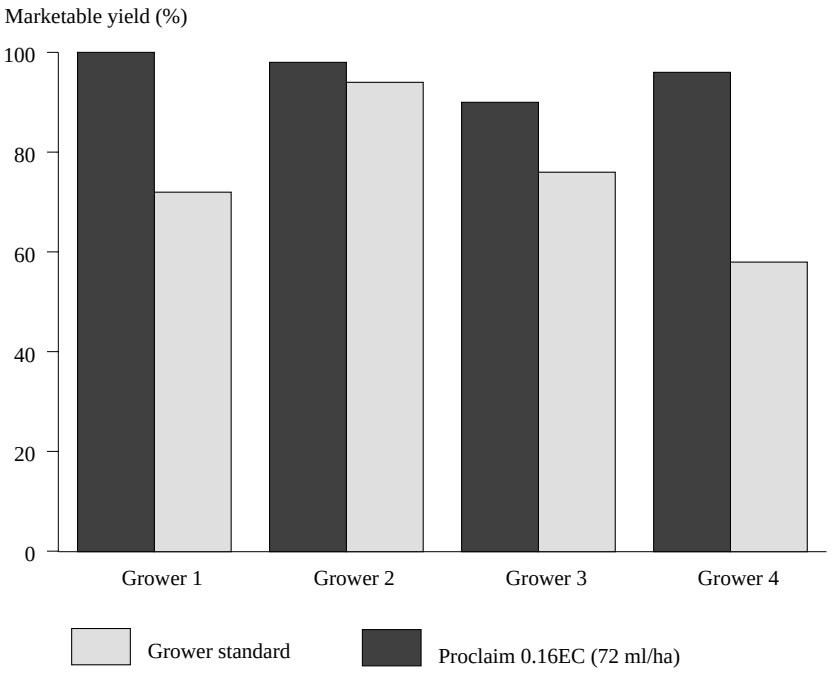


Figure 3b. Evaluation of PROCLAIM 0.16 EC by commercial growers in Hawaii. Comparison of harvest yields from PROCLAIM and growers standard treatment plots.

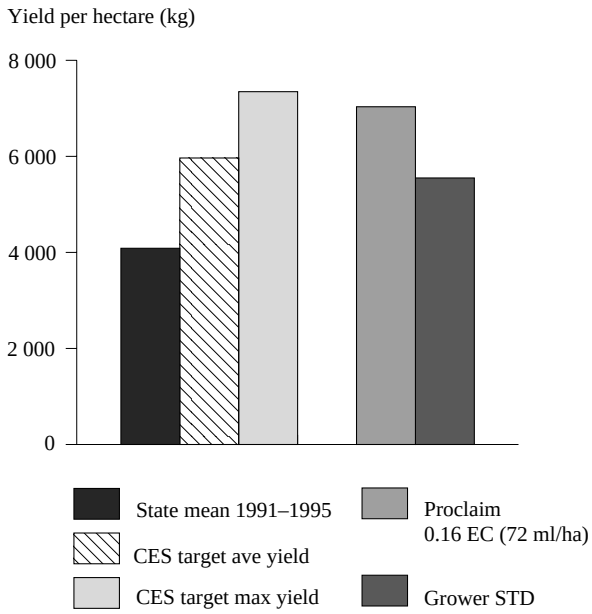


Figure 4. Comparison of grower cabbage yields with State of Hawaii and Cooperative Extension standard yields.

cabbage yields. The grower that surpassed extension's average yield made high insecticide expenditures by spraying twice each week for practically the entire crop cycle.

Discussion

The present Bta insecticides that growers use, Xentari and Mattch Bio-insecticide, gave varying rates of control. Although they were the most effective U.S. EPA registered DBM larvicides, the products performed best at lower DBM population densities and did not come close to providing adequate levels of control during periods when population levels were highest. Taking this into account, University of Hawaii Cooperative Extension recommended their use in combination with DBM adulticides (mevinphos and naled) with the Bta larvicides. Early evening applications of either mevinphos or naled in combination with one of the Bta insecticides reduced egg deposition by killing the adults and reduced larval damage. This practice generally gave better control than with the Bta insecticides alone, but many growers did not follow the recommendation. To complicate matters, the use of mevinphos was banned in November 1995.

In contrast, emamectin benzoate 0.16 EC and 5 SG provided superior control of DBM and other Lepidopteran larvae at low as well as at very high population levels. Both formulations were equally effective. Although the grower validation trials involved the use of only the emulsifiable concentrate formulation, we found no reason to expect that the soluble granule formulation would not perform as well.

The use of PROCLAIM® 0.16 EC under the EUP by the commercial growers resulted in an average increase in marketable yield of 29 percent and an estimated net increase in gross revenues of about $660 per ha. Yields were particularly impressive when they were compared with the State's average yield and Cooperative Extension target yields *(Figure 4)*. Grower satisfaction of the new product was very high. They felt that it was very easy to use and that the results were predictable.

As a commercial product, emamectin benzoate will fit well into the present DBM management program. It has positive characteristics for use in an IPM program. Its rapid degradation on leaf surfaces provides a good margin of safety for parasitoids of DBM and other pest species. The rapid toxicity and long residual activity within the leaf tissue should provide excellent protection against larvae of DBM and other cruciferous Lepidopterans.

Although the outlook for DBM management in Hawaii is positive, there is a potential downside to registration of the product. Due to the lack of other highly effective products, we can expect that growers might rely only on this product and select for genetic resistance. We have no knowledge about the propensity for resistance development, but have taken action to reduce the use of emamectin benzoate. Hawaii Cooperative Extension entomologists devised a DBM management program that involved the use of a tolerant cabbage cultivar (Scorpio) and the application of insecticide combinations to reduce adult and larval densities. Growers were advised to make weekly Bta treatments in combination with mevinphos or naled. The applications were made immediately after sunset to have the greatest impact against adults.

A resistance management protocol was mandated when the industry in the State of Hawaii was granted a emergency exemption for use of PROCLAIM® 5 SG on cabbage in June 1996. Until other highly effective products become available, we will attempt to manage resistance by integrating cultural controls with judicious use of PROCLAIM® 5 SG and Bta products. Growers are encouraged to plant varieties such as cv. Scorpio that seem to be more tolerant to DBM than others. Insecticidal control of DBM during the first half of the season is limited to Bta or other insecticide products unless DBM larval densities exceed 1 larva per plant. PROCLAIM® 5 SG can be used if this threshold is exceeded, but its use is normally limited during the second half of the crop season. To limit unnecessary use, treatments are generally governed by a 0.5 DBM larva per plant treatment threshold except during the last two weeks prior to harvest when marketability can be greatly reduced at even low DBM densities.

References

Dybas, R.A., N. J. Hilton, J.R. Babu, Ranz A. Preiser, and G. J. Dolce (1989). Novel second-generation avermectin insecticides and miticides for crop protection. *In:* Novel Microbial Products for Medicine and Agriculture. A. L. Demain, G.A. Somkuti, J. C. Hunter-Cevera and H.W. Rossmoore [eds]. pp 203–212.

Dybas, R.A. and J. R. Babu (1988). 4"-deoxy-4"-methylamino-4-epiavermectin B1 hydrochloride (MK243): A novel avermectin insecticide for crop protection. Brighton Crop Protection Conference. pp 57–64.

Lasota, J.A. and R.A. Dybas (1991). Avermectins, a novel class of compounds: Implications for use in arthropod pest control. *Annu. Rev. Entomol.* 36:91–117.

Tabashnik, B.E., N. L. Cushing, N. Finson and M. W. Johnson (1990). Field development of resistance in *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). *J. Econ. Entomol.* ***83:*** 1 671–76.

Tabashnik, B.E., N. Finson, J. M. Schwartz, M. A. Caprio and M. W. Johnson (1992). Diamondback moth resistance to *Bacillus thuringiensis* in Hawaii. pp. 175–183. *In:* N.S. Talekar [ed.], Proceedings of the second international workshop on the management of diamondback moth and other crucifer pests. Asian Vegetable Research and Development Center, Tainan, Taiwan.

Mau, R.F.L., L. Gusukuma-Minuto, and W. S. Ruggero (1995a). Field evaluation of bio-rational insecticides and combinations for controlling DBM, 1993. *Arthropod Management Tests,* ***20:*** 73–74.

Mau, R.F.L., L. Gusukuma-Minuto, W. S. Ruggero and R. Shimabuku (1995b). Field evaluation of a neem and Bt product for DBM control, 1994. *Arthropod Management Tests,* ***20:*** 74–75.

Mau, R.F.L., L. Gusukuma-Minuto, W. S. Ruggero and R. Shimabuku (1995c). Field evaluation of bio-rational insecticides for controlling DBM, 1993. *Arthropod Management Tests,* ***20:*** 75–76.

Mau, R.F.L., L. Gusukuma-Minuto, and R. Shimabuku (1995d). Laboratory evaluations against bioinsecticides against DBM larvae, 1994. *Arthropod Management Tests,* ***20:*** 327–328.

DICARE^{R} WG37.5 as a partner of anti-resistance strategy programme for the control of diamondback moth (*Plutella xylostella* L.) in Thailand

Jaruek Ribuddhachart[1] , Ittidet Chaimongkol[1], Patiparn Saitarnthong[2] , Somsith Chomenansilpe[2] and Amornrut Bhandhufalck[2]

[1]*R&D Insect Control, Ciba R&D Station, Tak Fah, Nakhon Sawan, Thailand*

[2]*Crop Protection Technical, Ciba-Geigy (Thailand) Limited*

Abstract

DICARE^{R} WG37.5 is a mixture of POLO^{R} (diafenthiuron) and INSEGAR^{R} (fenoxycarb). POLO^{R} is a representative of a novel chemical class of insecticide, highly active on *Plutella xylostella* at 50 g ai/hl, whilst INSEGAR^{R} (fenoxycarb) is a new juvenile hormone analogue with typical growth regulating features, especially interference with the last larval moult and, or ovicidal activity against many lepidopterous pests, at 10–15 g ai/hl.

In Thailand, resistance development of *P. xylostella* and *Spodoptera exigua* remains a critical factor in shortening the product life cycle of efficacious insecticides, as farmers repeatedly apply effective products. Usually the two pests are observed in a mix population, damaging/destroying cruciferous crops. If an insecticide is only effective on one of these pests,the farmer would mix/cocktail with other insecticide in order to control both pests.

Following laboratory test during 1994–1995, DICARE^{R} 37.5WG, was then evaluated in the farmers' fields under practical use conditions.

Under laboratory test, DICARE^{R} (300 ppm POLO^{R}+100 ppm INSEGAR^{R}) was very effective against both the egg stage and the last instar larvae of *P. xylostella.*Whilst under field conditions, DICARE^{R} (30 g ai/hl POLO^{R}+7.5 g ai/hl INSEGAR^{R}), proved effective against *P. xylostella* on Chinese kale. In addition, alternate application of DICARE^{R} with RAMPAGE (chlorfenapyr) clearly out performed all other treatments, thus proving that DICARE^{R} , applied alternately with other effective insecticides, not only provides best control of *Plutella xylostella* but also can be applied in an IRM programme to further prolong the life cycle of effective products in the market.

In conclusion, IRM should continue to be strongly promoted, developed as an effective tool for a better control of *P. xylostella* and other lepidopterous pests in Thailand and other countries.

Key words: Diamondback moth, DICARE, mixtures, insecticide resistance management

Introduction

Diamondback Moth (DBM), *Plutella xylostella* (L.) (Lepidoptera : Yponomeutidae) is one of the most serious insect pests of cruciferous crops all over the world (Hill, 1975). In Southeast Asia, it is a serious pest of cabbage, Chinese kale, Chinese cabbage, leaf mustard, Chinese radish and cauliflower. In Thailand, diamondback moth is generally prevalent from February to April when optimum climatic conditions and food plants are more readily available. However, in many areas of the central plain where crucifers are planted all year-round, diamondback moth damage can be observed through out the year (Rushtapakornchai and Vattanatangum, 1984). Damage from diamondback moth has been prevented only by spraying of chemical insecticide. However, application in large quantity and repeat use of the same chemical is always threatened by the development of resistance in a short period of time (Miyata *et al*., 1986). In Thailand, the microbial insecticide, *Bacillus thuringiensis* (Bt) has been used for diamondback moth control since 1972. Most commercial Bt products available belong to HD-1, *var. kurstaki*, serotype 3a/3b. Field evaluation of these products in 1986 showed promising results in only the northern area but not in the central plains and a loss of the Bt's efficacy was observed if it is applied just before an irrigation (Rushtapakornchai and Vattanatungum, 1986).

Ciba-Geigy, as a chemical company, is interested to evaluate a new product, DICARE WG37.5 for the control of DBM in the Southeast Asian countries. DICARE WG37.5 is a mixture of POLO^{R} (diafenthiuron) and INSEGAR (fenoxycarb). POLO is a representative of a novel chemical class of insecticide, which is highly active on *P. xylostella* at 50 g ai/hl (hectoliter) (Streibert and Kaeding, 1994), whilst INSEGAR (fenoxycarb) is a new juvenile hormone analogue with typical growth regulative features, especially interference with the last larval moult and, or ovicidal activity against many lepidopterous pests, at 10–15 g ai/hl (Senn and Frischnecht, 1994). The evaluation were therefore carried out to determine the following:

(1) Efficacy of DICARE (POLO/INSEGAR) vs *P. xylostella* under laboratory conditions.

(2) Efficacy of DICARE (POLO/INSEGAR) vs *P. xylostella* under field conditions.
(3) Application strategy of DICARE as a partner in anti-resistance programme vs *P. xylostella* in comparison with the farmer's practices.

Materials and Methods

Laboratory conditions (Ciba-Geigy R&D Station, Tak Fah)

- ***For eggs test:*** 16 potted cabbage plants (1 month old) were placed in the mating cage in which 100 pairs of *P. xylostella* adult moths are released for 2 days to allow oviposition on the leaves. Remove the cabbage plants from the cage. POLO SC250 (300 ppm), INSEGAR WP25 (100 ppm) and a mixture of POLO+INSEGAR (300+100 ppm) were employed in the test.
  Total no. of treatments: 4, including untreated check
  Total no. of replicates: 4 (1 potted cabbage plant/ rep)
  Total cabbage seedlings for this test: 16
  – Number of eggs on the leaf surface were recorded
  – Chemical solutions, according to the treatment list were applied individually thoroughly the whole plant with hand spray (10 ml/plant).
  – Number of newly hatch larvae/plant were recorded
  – Number of larvae/plant at 3 days after application were recorded
  – % mortality was calculated

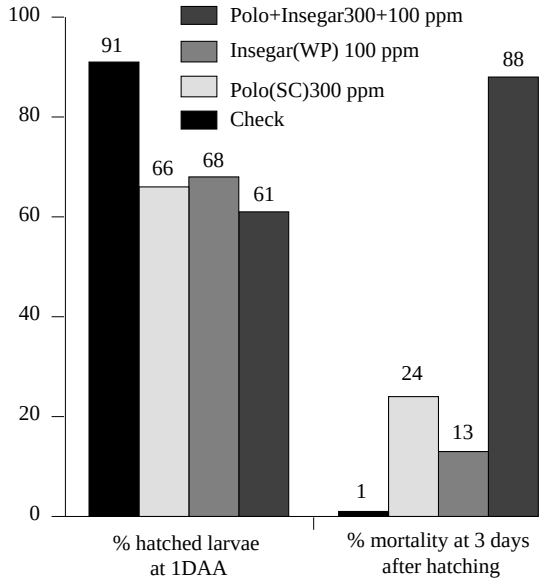


Figure 1. Effect of POLO, INSEGAR and POLO+INSEGAR on eggs of Plutella xylostella

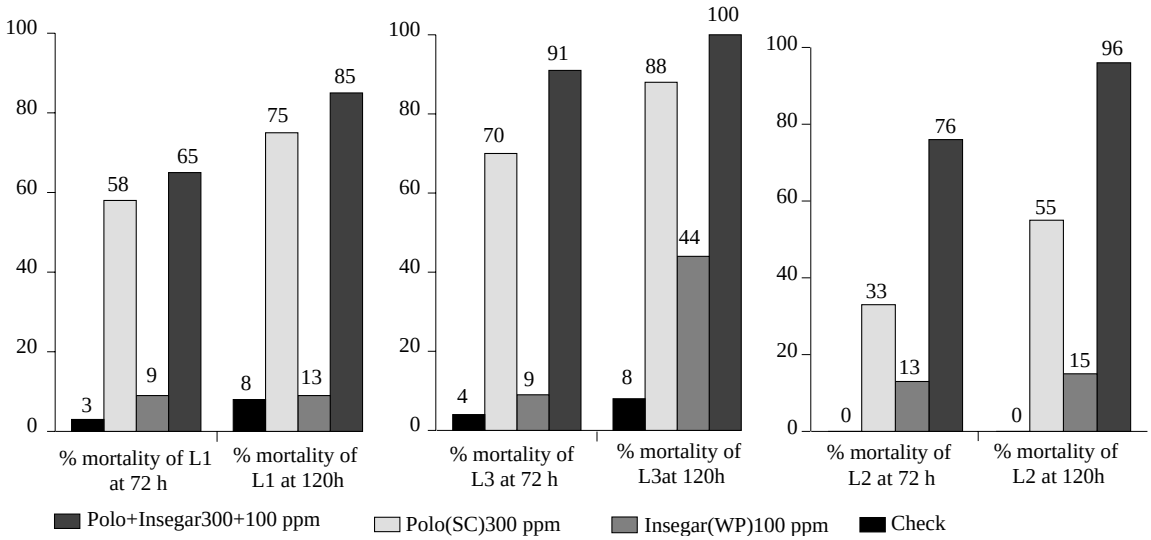


Figure 2. Effect of POLO, INSEGAR and POLO+INSEGAR on Plutella xylostella (L1), (L2) and (L3).

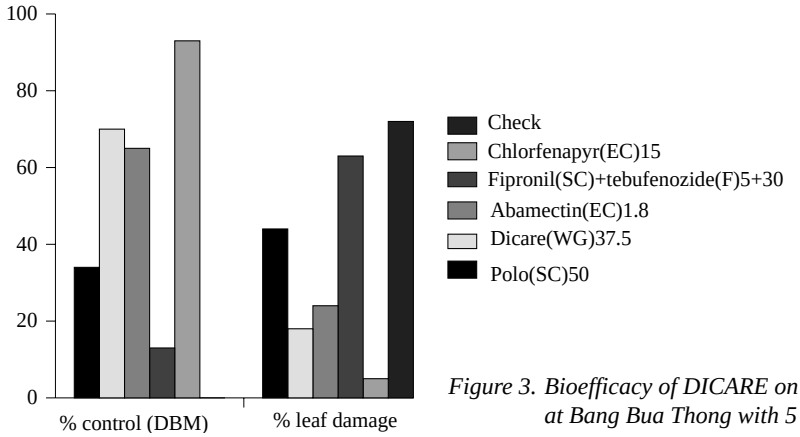


Figure 3. Bioefficacy of DICARE on Plutella xylostella on Chinese kale at Bang Bua Thong with 5 days intervals.

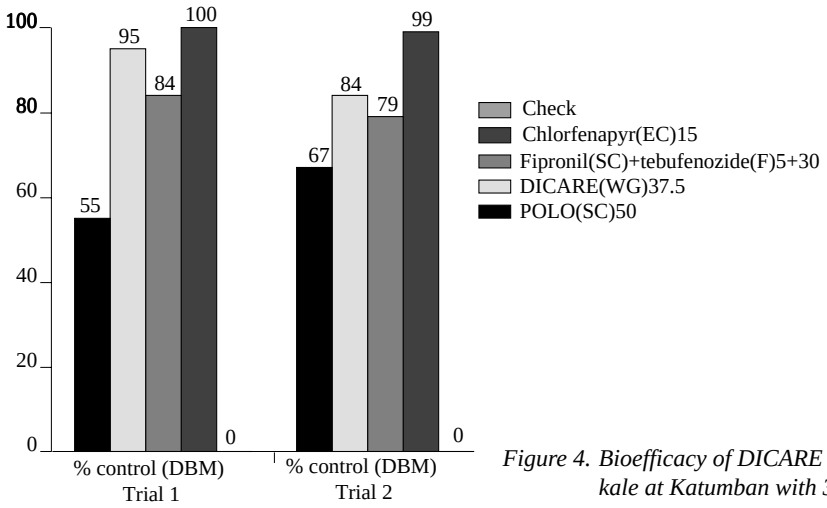


Figure 4. Bioefficacy of DICARE on Plutella xylostella on Chinese kale at Katumban with 3 days intervals

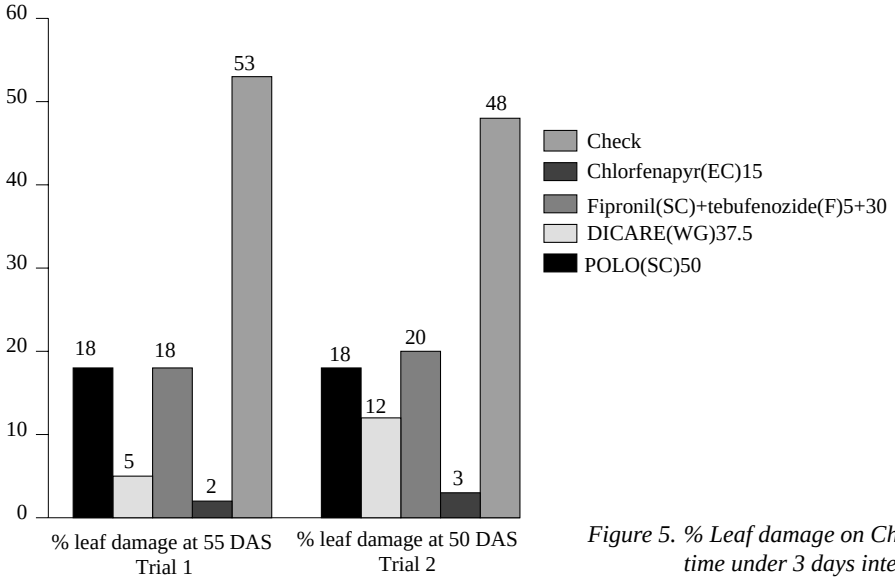


Figure 5. % Leaf damage on Chinese kale at harvesting time under 3 days interval at Katumban

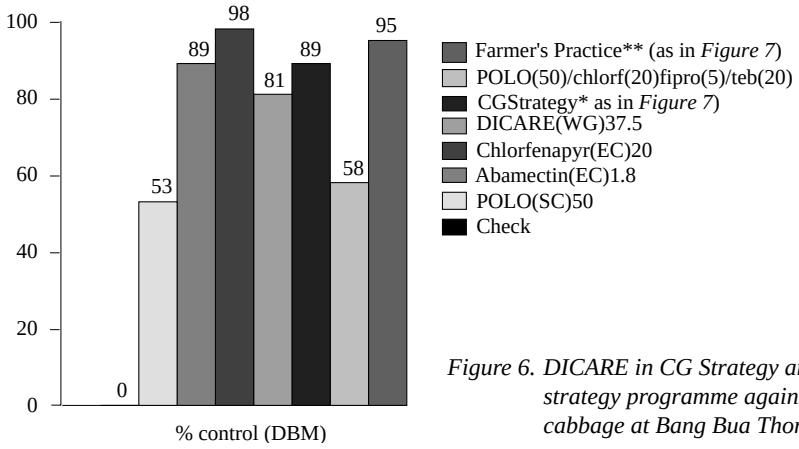


Figure 6. DICARE in CG Strategy and as a partner of anti-resistance strategy programme against Plutella xylostella on Chinese cabbage at Bang Bua Thong

- ***For larval test:*** Potted cabbage plant and chemical preparations were made in the same way as the egg test.
  - Chemical solutions were applied individually thoroughly the whole plant with hand spray, according to the treatment list (10 ml/plant)
  - The treated leaves were cut and put in the plastic box, accordingly.
  - Total no. of treatments: 4, including untreated check
  - Total no. of replicates : 4, 1 leaf/rep
  - 80 L1, 80 L2, 80 L3 (for 4 reps) were transferred to the treated leaves, according to the treatment list :check, POLO (300 ppm), INSEGAR (100 ppm) and POLO+INSEGAR (300+100 ppm), respectively.
  - Number of dead larvae at 3 and 5 days after application were recorded
  - % mortality was calculated
- ***Field trials:*** A suspension concentrate formulation of POLO (diafenthiuron), 250SC, INSEGAR (fenoxycarb) 25WP and a ready mixed formulation

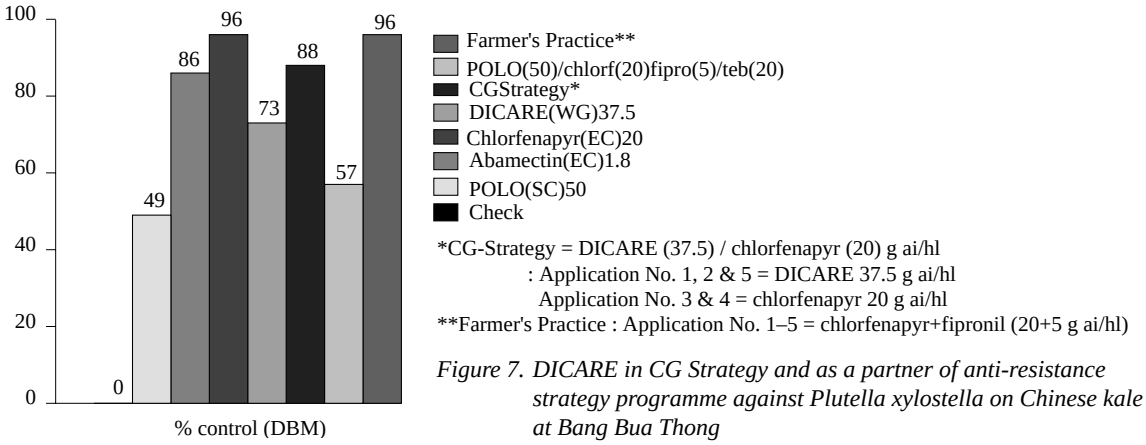


Figure 7. DICARE in CG Strategy and as a partner of anti-resistance strategy programme against Plutella xylostella on Chinese kale at Bang Bua Thong

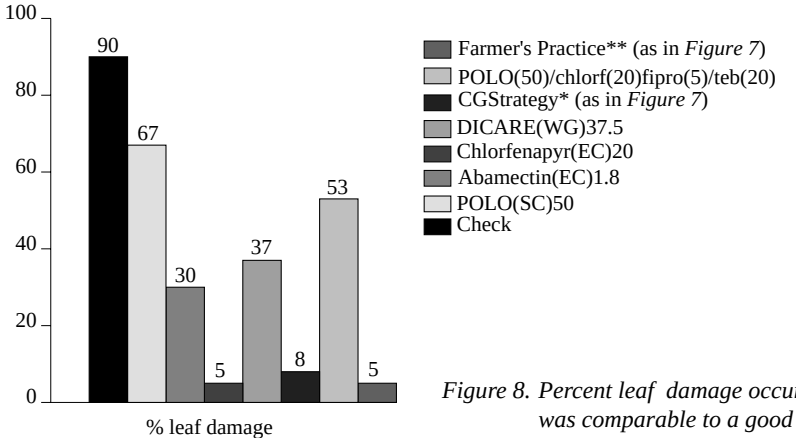


Figure 8. Percent leaf damage occurring in DICARE alternation strategy was comparable to a good standard, chlorfenapyr

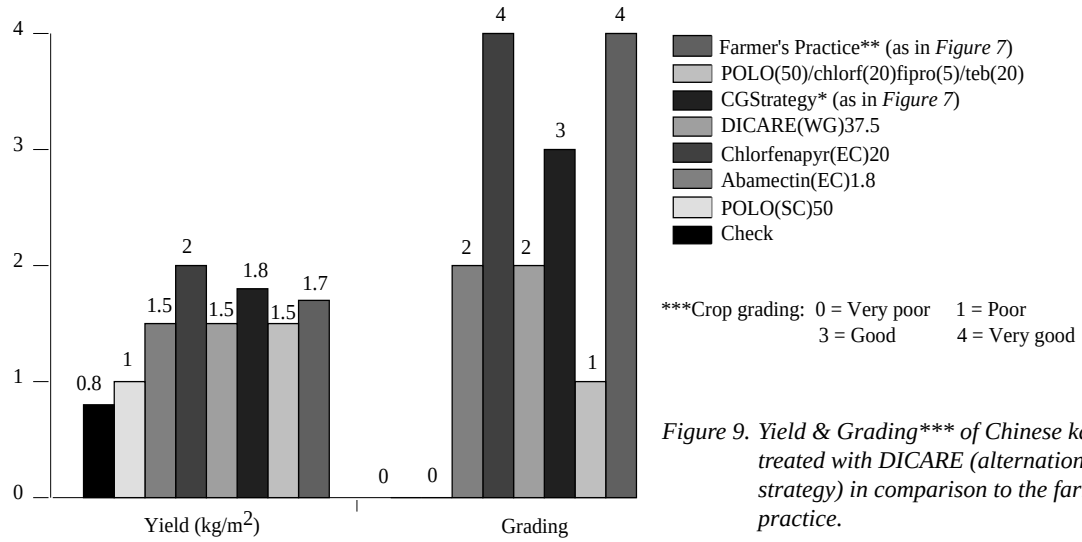


*Figure 9. Yield & Grading*** of Chinese kale treated with DICARE (alternation strategy) in comparison to the farmer's practice.*

of POLO & INSEGAR (DICARE 37.5WG) were evaluated in the field.A randomized complete block design was employed with 4 replicates (37.5 g.ai). The plot sizes varied from 10–20 $m^{2.}$

Foliar application of POLO (50 g.ai/hl), and POLO+INSEGAR, 30+7.5 or DICARE 37.5 g ai/ha with a spray volume of 1000 L/ha were made with local standard treatments and untreated check. The applications were timed on eggs, and young *P. xylostella* larvae (L1, L2 & L3). The first application was made when eggs was observed on the leaves or 2–3 weeks after sowing.

The assessments were made by counting the number of living larvae and pupae on 10–20 plants per replicate, and/or a final leaf damage rating at just before harvest. Marketable yield was also recorded.

Results & Discussion

- ***Under laboratory condition***

Egg test: POLO+INSEGAR, 300+100 ppm showed superior ovicidal effect to either POLO or INSEGAR.Very high % mortality of the newly hatched larvae at 3 days (72 h) after application was also recorded in a POLO+INSEGAR treatment *(Figure 1).*

Larval test: POLO+INSEGAR, 300+100 ppm showed superior larvicidal effect than either POLO

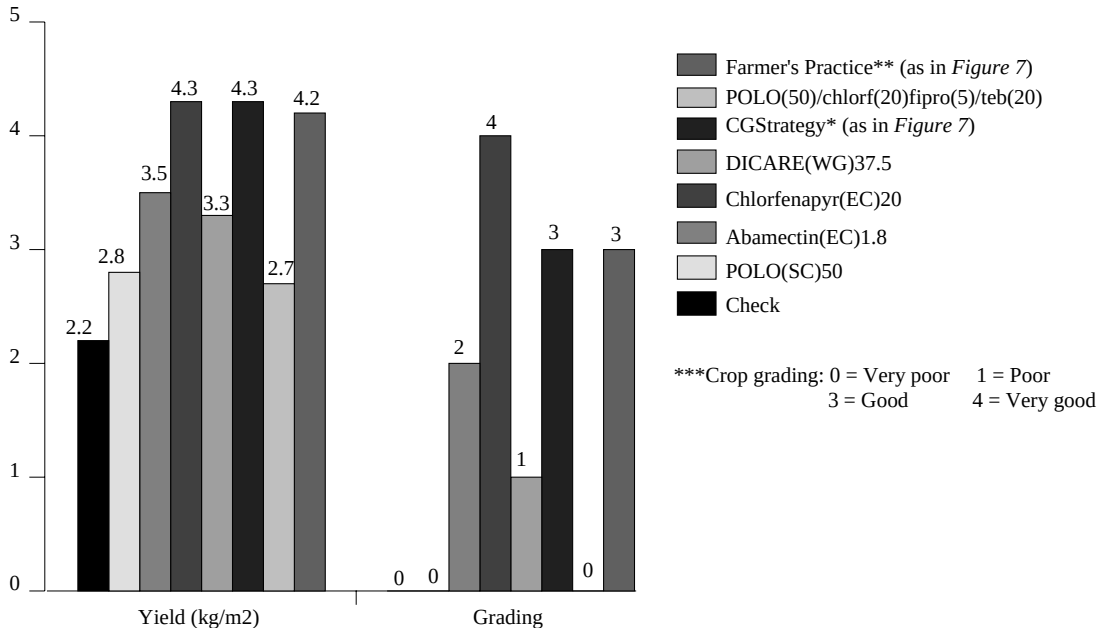


Figure 10. Yield & Grading of C. cabbage treated with DICARE (alternation strategy) in comparison to the farmer's practice*

or INSEGAR.Very high % mortality of the L3 larvae at 5 days (120 h) after application was also recorded in a POLO+INSEGAR treatment *(Figure 2)*.

- ***Field trials***

  At Bang Bua Thong (BBT) and Krathumban (KBT), DICARE^{R} 37.5 g ai/hl at 5 days intervals with a spray volume of 1000 L/ha was effective against *P. xylostella* and superior to abamectin, 1.8 g ai/hl and fipronil+tebufenozide, 5+30 g ai/hl. However DICARE was inferior to chlorfenapyr, 15 g ai/hl. *(Figure 3)*.

  When DICARE, 37.5 g ai/hl was applied at 3 days intervals, the bioefficacy on *P. xylostella* increased and comparable to chlorfenapyr, 15 g ai/hl. *(Figures 4* and *5)*. B. DICARE as a partner in an anti-resistance strategy programme against *P. xylostella.*

  Trials were conducted at Bang Bua Thong, in comparison with farmer's practices.The results showed that DICARE (37.5 g ai/hl) applied right at the beginning and in alternation with chlorfenapyr 20 g ai/hl gave good control of *P. xylostella*. Percent *P. xylostella* control and the marketable yield were taken into account *(Figures 6, 7, 8, 9* and *10).*

Conclusions & Recommendations

DICARE^{R} (POLO+INSEGAR) at 300 ppm+100 ppm under laboratory condition, and at 37.5 g ai/hl under field condition showed enhancement of activity in the control of *P. xylostella*. Besides, DICARE can be considered as an ideal partner in an anti-resistance strategy programme for the control of *P. xylostella* and/or *Spodoptera exigua* in cruciferous crops. In order to maintain the effective life of an insecticide, the recommendations for use of DICARE on cruciferous crops are as follows:

1) Do not apply DICARE repeatedly.
2) Alternate DICARE with other *P. xylostella* effective products
3) DICARE should be applied right at the beginning of *P. xylostella* infestation (oviposition period).
4) Apply only 2 applications of DICARE/crop.

References

Hill, P.S. (1975). Agricultural Insect Pests of the Tropic and their control. Cambridge University Press, London. 500 p.

Miyata, T., T. Saito and V. Noppun (1986). Studies on the mechanism of diamondback moth resistance to insecticides, p. 347–357. *In:* T.G., Griggs and N.S. Talekar (eds). Diamondback Moth Management. AVRDC, Taiwan.

Rushtapakornchai, W. and A. Vattanatungum (1984). Comparison study on the efficacy of certain insecticides for control lepidopterous larvae on Chinese cabbage. Annual Research Report for 1984. Entomology and Zoology Division, Department of Agriculture, Bangkok, Thailand.

Rushtapakornchai, W. and A. Vattanatungum (1986). Present status of insecticide control of diamondback moth in Thailand, p 307–312. *In:* T.G., Griggs and N.S. Talekar (eds.). Diamondback Moth Management. AVRDC, Taiwan.

Streibert, H.P., and D. Kaeding (1994). Use of Pegasus^{R} (diafenthiuron) against diamondback moth (*Plutella xylostella*) and other lepidopterous pests in cruciferous crop. Crop Protection Division, Ciba-Geigy AG, CH-4002 Basle, Switzerland, 6 p.

Senn R., and M.L. Frischnecht (1994). Use of fenoxycarb to control the citrus leaf miner (*Phyllocnistis citrella*) (Stnt).Crop Protection Division, Ciba-Geigy AG, CH-4002 Basle, Switzerland, 4 p.

AC303,630 – A new novel insecticide-acaricide for control of resistant arthropod pests

S. C. Lee[1], C. Sujin[2], P. J. Huang[3], X. M. Zhang[4] and G. T. Ooi[5]

[1]*Cyanamid International, Singapore*
[2]*Cyanamid (Thailand) Ltd., Bangkok, Thailand*
[3]*Cyanamid Taiwan Corporation, Taipei, Taiwan*
[4]*Cyanamid (China) Co.Ltd., Shanghai, People's Republic of China*
[5]*Tiram Crop Protection Sdn. Bhd., Kuala Lumpur, Malaysia*

Abstract

AC303,630 (chlorfenapyr), a member of a new class of compound known as the pyrroles, is a broad spectrum insecticide/acaricide on many economically importance crops. AC303,630 is highly active by ingestion, posesses contact activity and provides moderate residual activity on plants. Extensive field study in many Asian countries has shown AC303,630 to be highly effective on many economically important arthropods which have shown resistance to conventional classes of insecticides. Examples of pests that are effectively controlled by AC303,630 include *Spodoptera exigua, Plutella xylostella, Heliothis virescens, Thrips palmi* and *Tetranychus* spp. AC303,630 can be a promising and cost effective candidate for use in resistance management programmes in high value crops.

This paper describes the bioactivity of AC303,630 on insect pests found on economically important crops in selected Asian countries.

Key words: Chlorfenapyr, pyrolles, insect pests, resistance management

Introduction

AC303,630 (Trademark: Rampage, Secure, CHU-JIN) is a new broad spectrum insecticide-acaricide, belonging to the pyrroles chemical group discovered and developed by American Cyanamid Company. Miller *et al.* (1990), reported that AC303,630 provided commercially acceptable control of 35 insect and mite pests important in crop production. Merriam *et al.* (1992), also characterized the insecticidal activities of AC303,630 against *Heliothis virescens* and *Plutella xylostella*.

This paper describes the field performance of AC303,630 for the control of certain insect, thrip and mite species which are known to exhibit resistance to various insecticides and acaricides.

Materials and Methods

The study on organophosphates, pyrethroid, carbamate and IGR-resistance of diamondback moth and beet armyworm has been well documented by research workers and scientists in Thailand (Dept. of Agriculture, 1992), the People's Republic of China (PRC), (Guangdong Prov PPI, 1993) and Taiwan (AVRDC, 1985), using the bioassay technique.

Results presented in this paper were obtained from in-house and cooperative field trials in various Asian countries on conventional class insecticide-resistant diamondback moth, beet armyworm, thrips and 2-spotted mites. Emulsifiable concentrate (EC) and the suspension concentrate (SC) formulations of AC303,630 (100 g ai/li EC or SC) were used in the trials. Foliar applications were made at rates ranging from 50 to 300 g ai/hectare, comparing with local standard treatments (chlorflurazuron and abamectin) applied at recommended rates and with untreated check plots.Three to four replicates were used in randomised complete block design. Insect population indices and damage ratings were determined by standard evaluation procedures which varied by insect species. Yield performance was assessed based on marketable yield quality and damage rating.

Results

Plutella xylostella (DBM)

In trials conducted in Thailand, Taiwan and the PRC on Chinese kale, common head cabbage and cauliflower, both the emulsifiable concentrate (10% EC) and the suspension concentrate (10% SC) at rates from 50 to 200 g ai/ha provided near complete control of 1–2 and 3–4 instar DBM larvae, and was superior to the standards abamectin and chlorfluazuron *(Figures 1, 2, 3* and *4)*. All treatments were applied 2–7 times at 5 to 10-day intervals.

The 75–150 g ai/ha rates were consistently comparable or superior to abamectin or chlorfluazuron.

Spodoptera exigua (Beet armyworm)

Figures 5, 6, and *7* demonstrate the excellent performance of AC303,630 on beet armyworm on Chinese kale and common head cabbage. All doses of AC303,630 tested were superior to abamectin (18 g ai/ha). The degree of residual control was directly correlated with AC303,630 application rates. The 100–150 g dose range appears optimum to provide effective control of this pest which has shown to be increasingly difficult to manage.

At harvest, all AC303,630 treatments were providing sufficient DBM and beet armyworm control

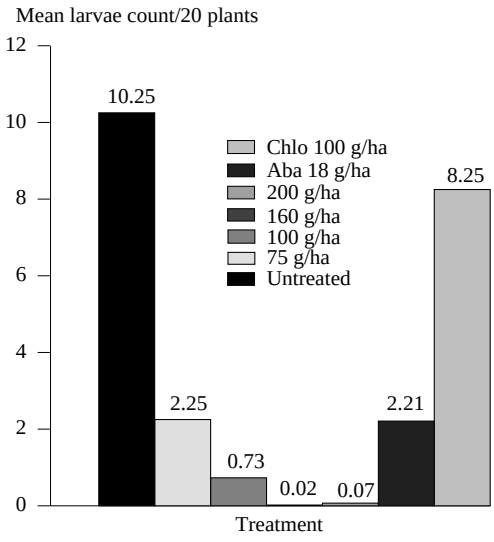


Figure 1. AC 303,630 SC/EC efficacy – Thailand diamondback moth, Chinese kale, 1990–91

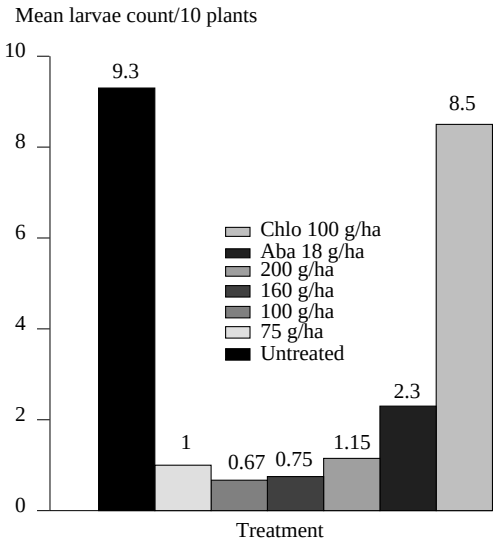


Figure 2. AC 303,630 SC/EC efficacy – Thailand diamondback moth, Chinese cabbage, 1992

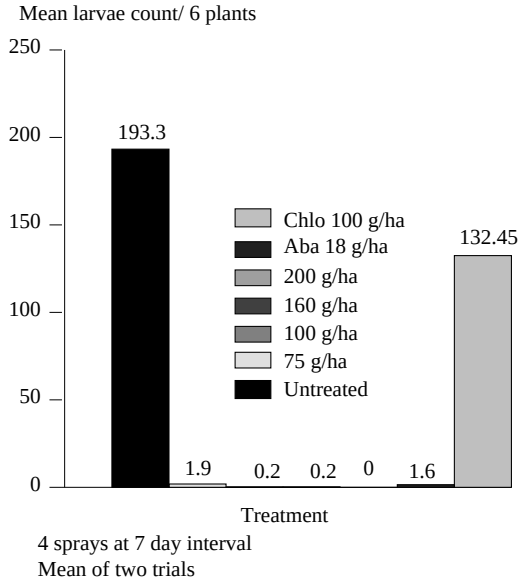


Figure 3. AC 303,630 SC/EC efficacy – Taiwan diamondback moth, Cauliflower, 1990–91

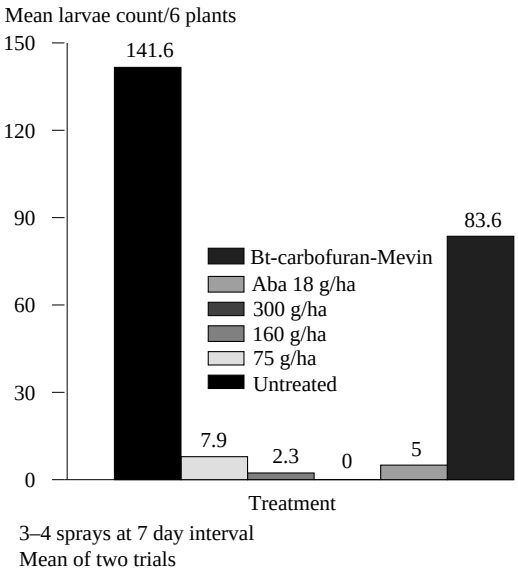


Figure 4. AC 303,630 SC/EC efficacy – Taiwan diamondback moth, Common cabbage, 1990–91

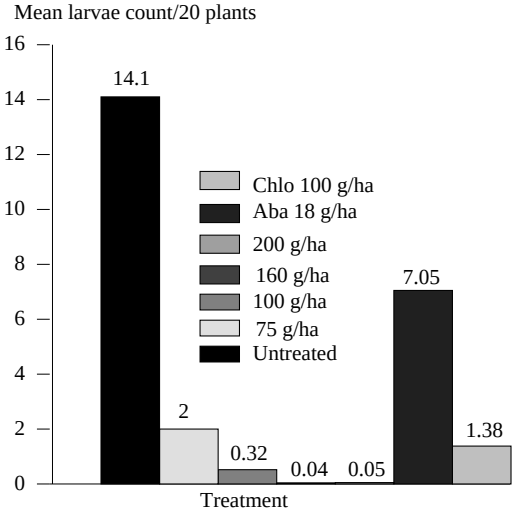


Figure 5. AC 303,630 SC/EC efficacy – Thailand beet armyworm, Chinese Kale, 1990–91

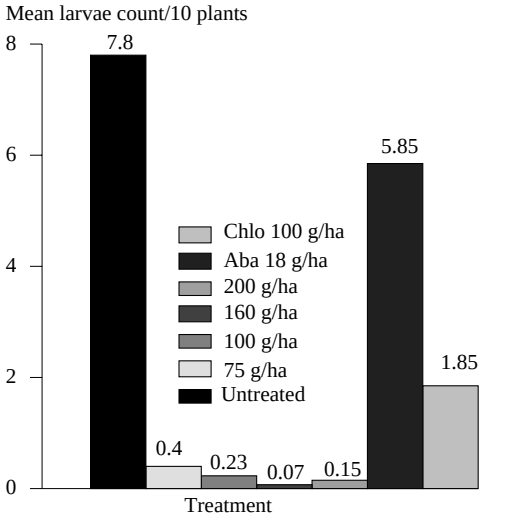


Figure 6. AC 303,630 SC/EC efficacy – Thailand beet armyworm, Chinese Cabbage, 1990–91

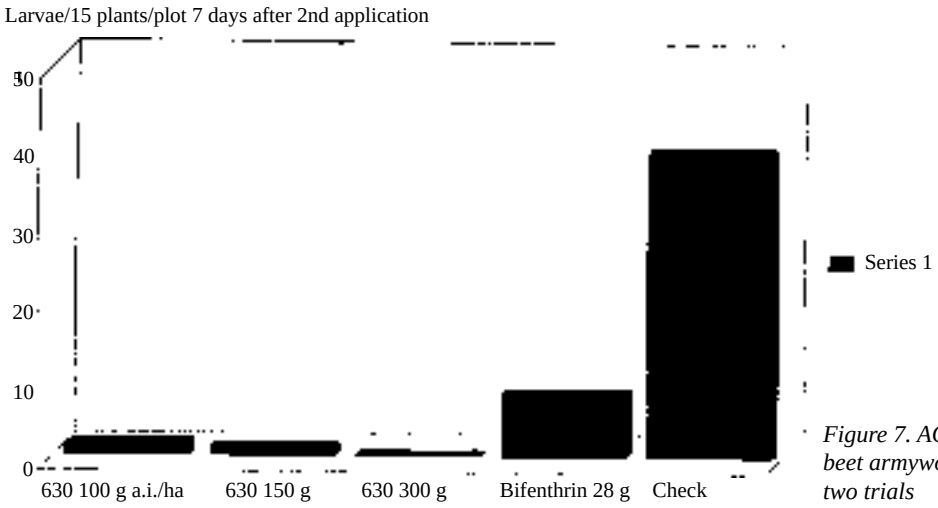


Figure 7. AC303,630 10SC/maize beet armyworm, Taiwan-Mean of two trials

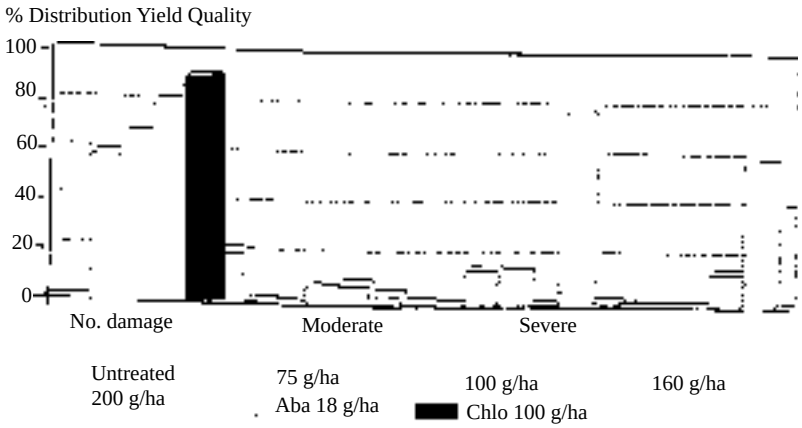


Figure 8. AC303, 630 SC/EC efficacy – Thailand yield performance, Chinese kale, 1990–91

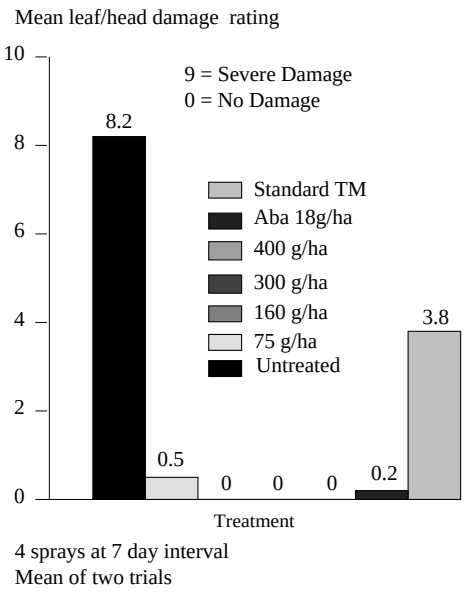


Figure 9. AC 303,630 SC/EC efficacy – Taiwan yield performance, cauliflower, 1990–91

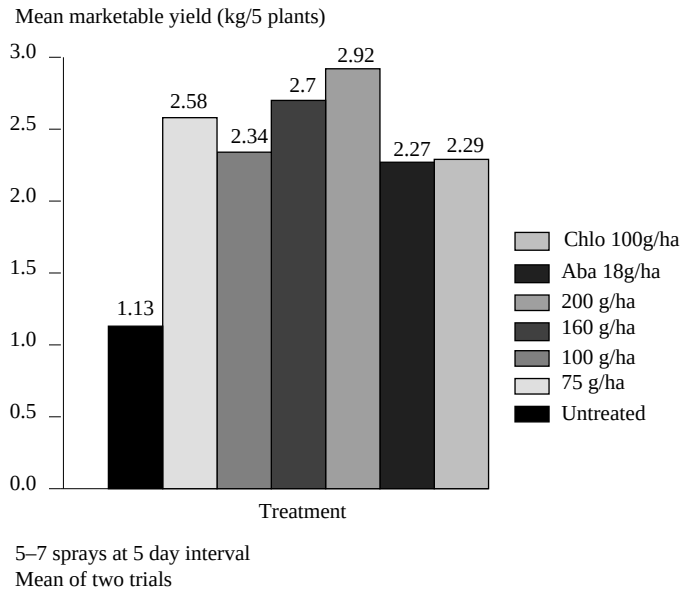


Figure 10. AC 303,630 SC/EC Efficacy – Thailand yield performance Chinese cabbage, 1992

to allow for a high percent distribution of yield (Chinese kale) in the grade of "No Damage", ranging from 60% (75 g ai/ha) to >85% (200 g ai/ha), *(Figures 8* and *9)*. Avermectin provided only 20% yield with "No Damage" which was significantly less than that proivided by any AC303,630 treatment. Yield quality ratings in the chlorflurazuron (100 g ai/ha) and the untreated plot were significantly low with close to 60% and 80% "Severe" feeding damaged leaves. *(Figures 10* and *11)*

All rates of AC303,630 were clearly enhancing superior yield, be it on Chinese kale, common head cabbage or cauliflower.

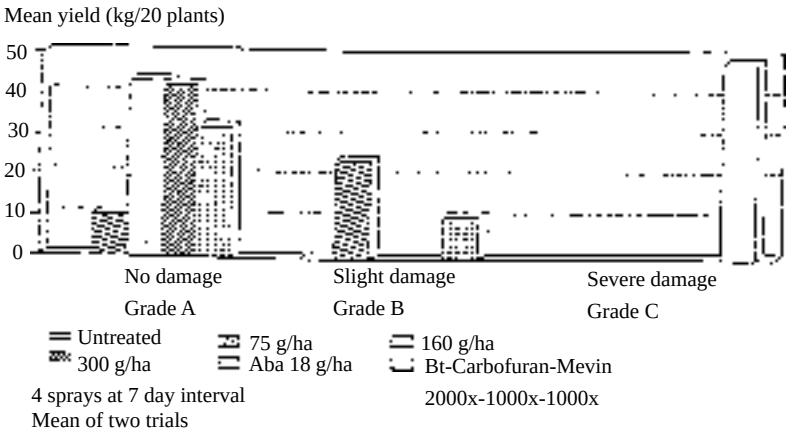


Figure 11. AC 303,630 SC/EC efficacy – Taiwan yield performance, common cabbage, 1990–91

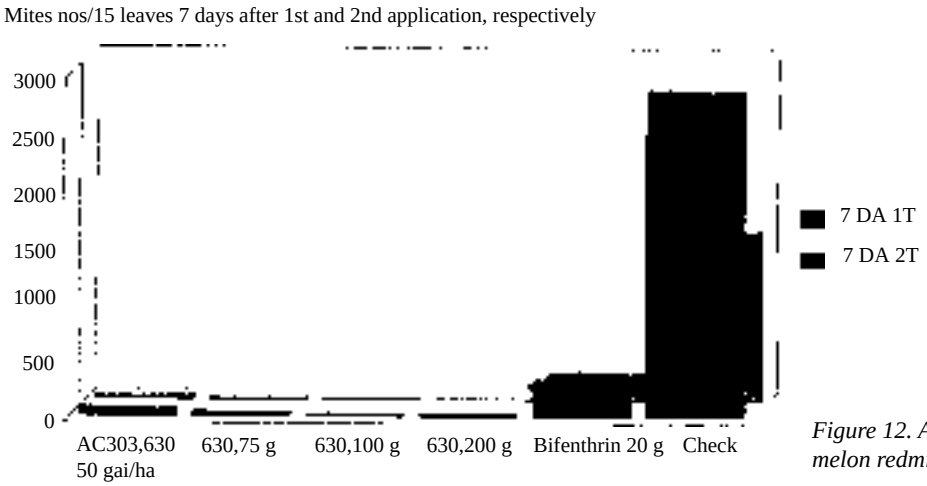


Figure 12. AC303, 630 10SC/water melon redmites, Taiwan, 1993

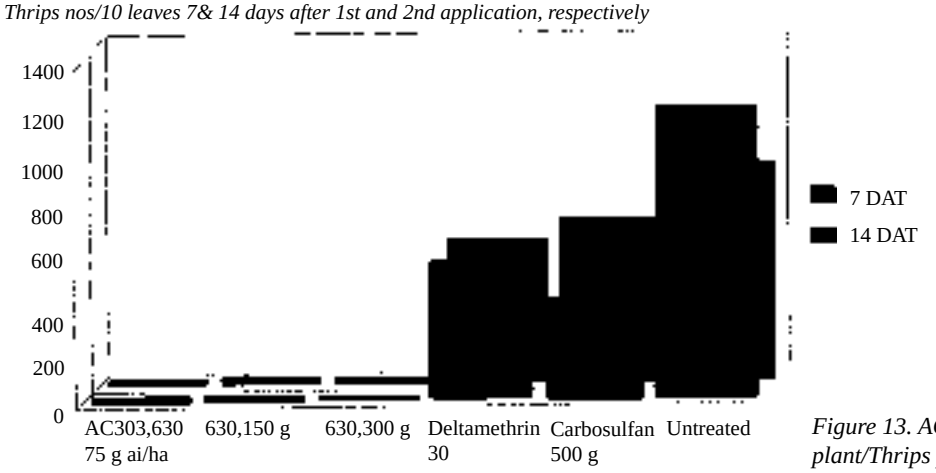


Figure 13. AC303,630 10EC/egg plant/Thrips palmi, Taiwan 1993

***Tetranychus urticae* (2-spotted red spider mite)**

In trials conducted in Taiwan in 1993, AC303,630 10%EC at rates from 50–200 g ai/ha, using a 7-day interval spray regime had provided excellent fast knock-down of the mite population. *Figure 12* shows the rapid reduction in number of the mites in all AC303,630 treated plots versus standards and the untreated check 7 days after the 1st and 2nd application. Bifenthrin at 50 g ai/ha failed to provide adequate control in watermelon.

Thrips Species

In egg-plant trials in Taiwan, AC303,630 at 75 to 300 g ai/ha provided excellent control of the pest in terms of thrip population reduction following 2 applications at weekly spray interval. *Figure 13* shows the reduction in numbers of *Thrips palmi* larvae in AC303,630 treated plots. The two standards deltamethrin and carbosulfan gave only marginal reduction of the thrips population.

In Japan, AC303,630 10SC at 150 g ai/ha provided excellent initial (86%) and residual control (97 and 94% at 7 and 14DAT, respectively) of a high population *T. palmi* on eggplant. *Figure 14* shows the

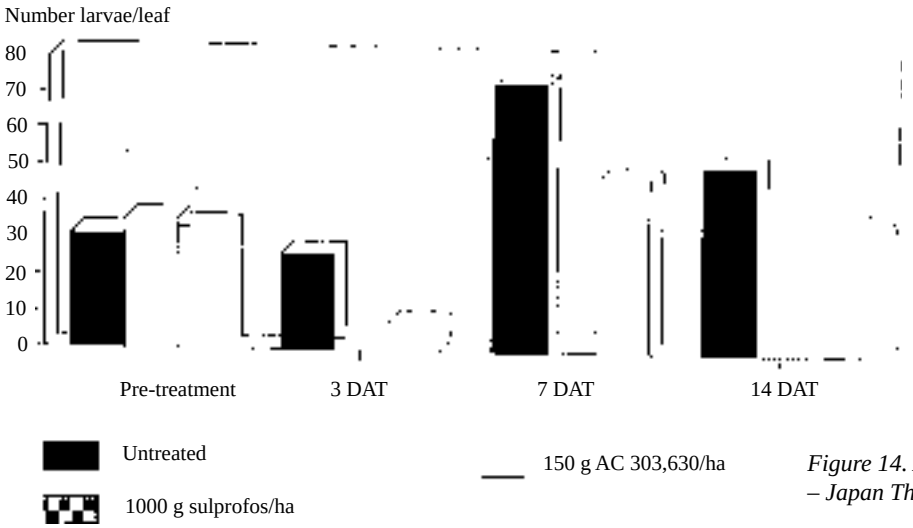


Figure 14. AC 303,630 10 SC efficacy – Japan Thrips palmi, eggplant, 1991

reduction in number of *T. palmi* larvae in the AC303,630 treated plots. Sulprofos 50EC at 1 000 g ai/ha reduced the number of *T. palmi* larvae/leaf to 7.7 at 3 DAT, but failed to provided sufficient residual control(only 33 to 39% control).

Conclusion

Results from extensive field trials in the Far East have shown AC303,630 at the tested dosages to be effective for control of many economically important arthropod species which have been reported to be resistant to various classes of insecticides and acaricides, some of which were mentioned in this paper under "Materials and Methods". These results suggest that AC303,630 can be an effective, useful alternative and integral part of resistance management programmes.

References

T.L. Merriam, R. Farlow, J. Beam, S.C. Lee and T.W. Homan (1992). Efficacy of AC303,630 Insecticide – Acaricide for control of resistant arthropod pests. *In:* Proceeding XIX International Congress of Entomology, Beijing 1992, China.

Miller, T.P., M.F. Treacy, I.E. Gard, J.B. Lovell, D.P. Wright, Jr., R.W. Addor and V.M. Kamki (1990). AC303,630 – Summary of 1988-89 field trial results. *In:* Proceedings Brighton Crop Protection Conference – Pests and Diseases. British Crop Protection Council, Brighton, Sussex, England 43–48.

Asian Vegetable Research and Development Center (1985). Diamondback Moth Management. Proc. of First International Workshop, Tainan, Taiwan. March 1985.

Department of Agriculture (1992). Report Meeting of the Joint Research Project. Insect Toxicological on Resistance to Insecticides in Diamondback Moth. Thailand.

Activity of fipronil on diamondback moth

Arlin L. Bostian, Sinchai Swasdichai, and Laili bin Darus
Rhône-Poulenc Secteur Agro Asia Pacific Zone
#27-02/05 The Concourse, 300 Beach Road
Singapore 679312

Abstract

Fipronil, a member of a new family of insecticides, was discovered by Rhône-Poulenc scientists in 1987 and placed into worldwide development in 1989. This insecticide is extremely active, often requiring only a few grams of active ingredient per hectare for control of piercing / sucking and chewing insect pests. Fipronil has proven to be particularly well suited for use on cruciferous vegetables as it possesses excellent activity on diamondback moth (DBM), *Plutella xylostella* L. In addition, fipronil also provides good control of imported cabbageworm, *Pieris* spp., and beet semi-looper, *Autographa nigrisigma*. Control is achieved primarily by ingestion, although contact activity can also be important. In more than seven years of field trials, fipronil has consistently demonstrated outstanding control of both susceptible and resistant DBM populations at application rates of 25–50 gai / ha. Fipronil provides long-lasting residual control of DBM, generally ranging from 7–14 days, and significantly improves marketable yields.

Biochemical and electrophysiological bioassays as well as extensive field use of fipronil have shown no cross-resistance with either carbamates, organophosphates, or pyrethroids. However, in view of the well-documented ability of DBM to develop resistance to virtually all commercially-used insecticides, Rhône-Poulenc in 1995 implemented a resistance monitoring program. This monitoring program included several Asian countries and, in 1996, was further expanded to include more locations within the participating countries as well as additional countries. While no indications of resistance development to fipronil have been detected in DBM to date, Rhône-Poulenc will continue to use this resistance monitoring program as a part of its overall strategy for carefully managing the use of fipronil with the objective of ensuring that the product remains an effective tool for the control of this important and damaging pest.

Key words: Fipronil, chemical control, diamondback moth (*Plutella xylostella* L.), resistance management.

Introduction

In 1981, Rhône-Poulenc scientists working at Ongar, U.K., discovered a new family of chemicals, unrelated to existing commercial insecticides. Fipronil, a member of this new class of insecticides known as the phenylpyrazoles, was discovered in 1987. Fipronil is an extremely active molecule, often requiring only a few grams of active ingredient per hectare to control piercing / sucking and chewing insect pests. The compound controls a broad spectrum of damaging insects and can be effectively delivered to the target pests via soil, foliar, bait, or seed treatment applications.

Like most insecticides, fipronil acts on the insect's central nervous system (CNS). By conducting numerous *in vitro* assays, Rhône-Poulenc biochemists and independent researchers have determined that fipronil interferes with the passage of chloride ions through the gamma-aminobutyric acid (GABA) regulated chloride channel. This interference disrupts normal CNS activity and, at sufficient doses, causes death. Furthermore, there is target site specificity between insects and mammals with fipronil displaying tighter binding (i.e., higher potency) in the insect GABA chloride channel than in the vertebrate, providing useful selective toxicity.

Due to its specific interaction with the GABA receptor, fipronil is considered to have a unique mode of action. Because of its unique mode of action and because it is from a new class of chemistry, fipronil has been shown to be effective against insect pests which have developed resistance to many conventional insecticides. This activity on resistant insects makes fipronil an excellent candidate for use in insect resistance-management programs.

Fipronil has both contact and ingestion activity and has demonstrated systemic activity in many crops, particularly when applied as a soil or seed treatment. Translaminar penetration and local systemic action also occur with foliar applications of fipronil. The rapid knock-down often associated with insecticides such as the pyrethroids is sometimes absent with fipronil and, in the field, insect mortality may appear to be somewhat slow. However, intermediate responses, such as cessation of feeding, may be noted soon after treatment. Residual control following foliar application is generally good to excellent.

In general, fipronil has minimal impact on beneficials primarily due to the low doses that are required for control of target pests, its spectrum of activity, and the insecticide's formulation and application flexibility. Comparisons made with

competitive products have confirmed this important advantage of fipronil and have underlined the product's excellent fit in integrated pest management (IPM) programs. Research trials conducted in several countries have shown that foliar applications of fipronil have little or no impact on beneficials such as spiders (*Lycosa* spp., *Tetragnatha* spp., *Erigonidium* spp., *Oedothorax* spp.) and predatory bugs (*Cyrtorhinus* spp.). Foliar applications, however, can be toxic to honeybees and care should be taken to time fipronil applications so as to coincide with a minimal presence of bees.

Materials and Methods

Biological efficacy: Following its discovery in 1987, initial screening trials with fipronil indicated exceptional activity against the diamondback moth (*Plutella xylostella* L.). From 1989 until its first registration for use on DBM in Thailand in 1994, numerous field trials were conducted in Asia as well as in other parts of the world. Several formulations have been tested including a suspension concentrate (SC) containing 50 grams active ingredient per liter, a 200 g ai/l SC, and an 800 g ai/kg water dispersible granule. Initial dose rates ranging from 10–100 g ai/ha were tested; fine-tuning resulted in emphasis on rates of 25–50 g ai/ha. Timing of the fipronil applications was typically 7–10 days; however, intervals as short as four days were tested as well as intervals longer than 10 days.

In Asia, field trials were conducted in accordance with local cultural practices. The product was generally applied using spray volumes from 250 to more than 1000 liters per hectare (usually, 250–600 liters/ha were used). While many different types of equipment were used, back-pack sprayers were by far the most common means of application. Field trials were normally replicated, but larger block, unreplicated trials were also used. Assessments included insect counts, damage ratings, total yield, and percent marketable yield. In all cases, the performance of fipronil was compared, "side-by-side" with that of the standard control practice. Insecticide standards compared with fipronil in these trials included (but were not limited to) abamectin, acephate, *Bacillus thuringiensis*, bendiocarb, carbosulfan, cypermethrin, chlorfenapyr, chlorfluazuron, fenpropathrin, fenvalerate, lambda-cyhalothrin, malathion, methamidophos, permethrin, pirimiphos-methyl, prothiofos, and teflubenzuron. All standards were used as recommended.

Resistance development / management: Biochemical and electrophysiological bioassays as well as extensive field use of fipronil have shown no cross-resistance with carbamates, organophosphates, or pyrethroids. In addition, in over two years of commercial use, no change in the field performance of fipronil on DBM has been documented. However, in view of the well-known ability of this insect to develop resistance to virtually all commercially used insecticides, Rhône-Poulenc has taken several steps intended to minimize the potential for resistance development. Among these actions was the implementation in early 1995 of the fipronil/DBM resistance monitoring program. This program included several countries from around the world, many of which were in Asia. In 1996, the monitoring program was further expanded to include more locations within the original participating countries as well as additional countries.

The procedure used in the determination of the susceptibility of DBM to fipronil is briefly as follows: Water is used to make serial dilutions of the formulated product (generally the 50 g ai/l SC formulation is used). These dilutions normally include concentrations of 0.03, 0.09, 0.3, 1.0, 3.0, 9.0, and 27.0 parts per million. Cabbage leaves are cut into strips approximately 1 x 2.5 cm and dipped in the respective fipronil concentrations (leaves dipped in water are used as controls). The treated strips of cabbage leaves are then placed into vials or small petri dishes and allowed to air dry. Two to five DBM second instar larvae are then introduced into each vial or petri dish with a minimum of four replications recommended for each concentration. The vials or Petri dishes are then closed and maintained at room temperature (20–25 °C). The larvae are observed daily for a period of three days and the percent mortality is recorded for each concentration. If necessary, an adjustment is made for any mortality noted in the controls. Using these results, LC_{50}'s are determined for each treatment by day. In general, DBM populations are monitored at least two times per year and the locations of the monitoring sites are maintained.

Results and Discussion

An overview of the results of these trials is presented in *Figures 1–6*. In over six years of field trials and two years of commercial use, fipronil has consistently demonstrated outstanding control of DBM. In replicated research trials, fipronil's performance (i.e., percent control of DBM larvae, persistence of activity,

Table 1. Efficacy of fipronil against other common lepidopterous pests of brassicas

Pest	Common name	Location	Activity at 25–50 g a.i./ha
Trichoplusia ni	Cabbage looper	Taiwan	Moderate
Trichoplusia ni	Cabbage looper	USA	Moderate
Pieris spp.	Imported cabbageworm	Taiwan	Excellent
Pieris spp.	Imported cabbageworm	USA	Excellent
Pieris spp.	Imported Cabbageworm	Japan	Good
Autographa nigrisigma	Beet semi-looper	Japan	Good
Spodoptera spp.	Armyworm	Taiwan	Poor

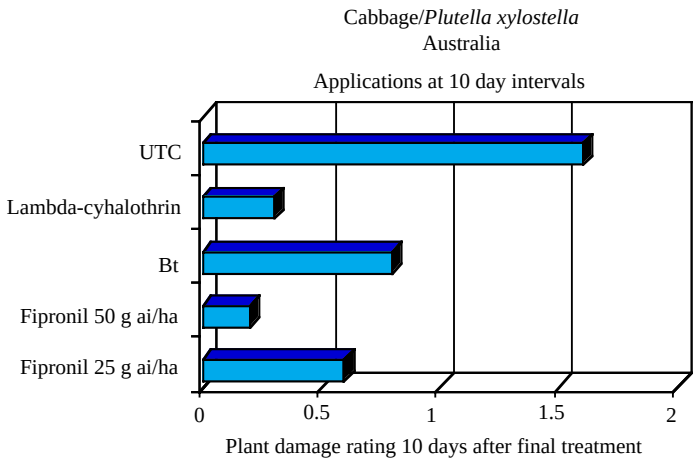


Figure 1. Efficacy of fipronil against Plutella xylostella on cabbage, Australia.

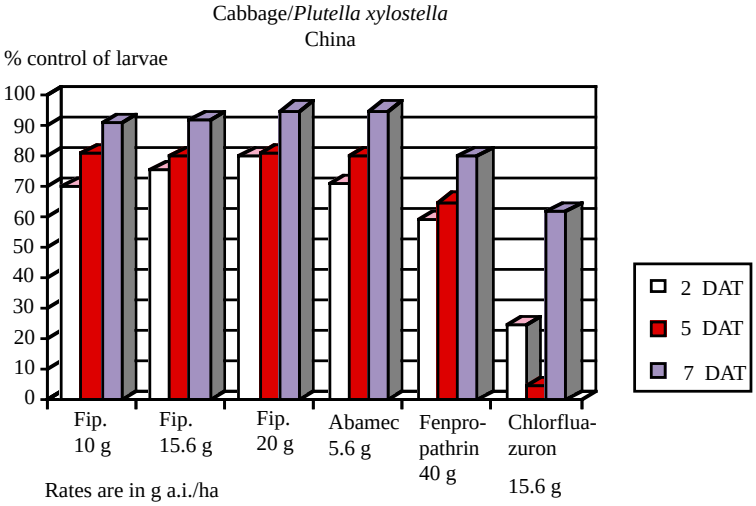


Figure 2. Efficacy of fipronil against Plutella xylostella on cabbage, China.

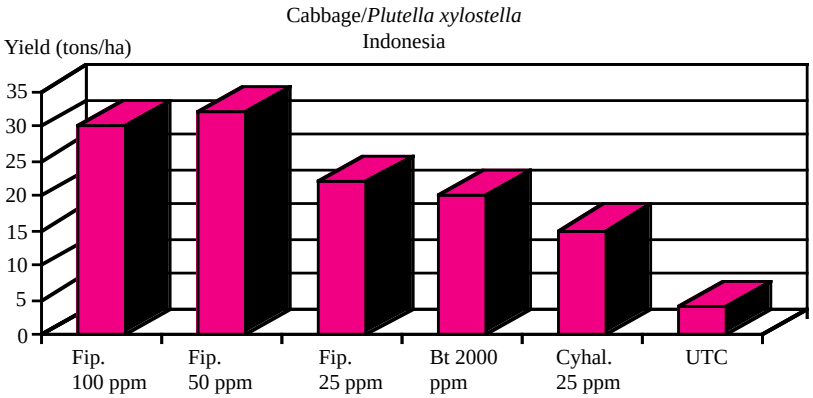


Figure 3. Efficacy of fipronil against Plutella xylostella on cabbage - yields, Indonesia.

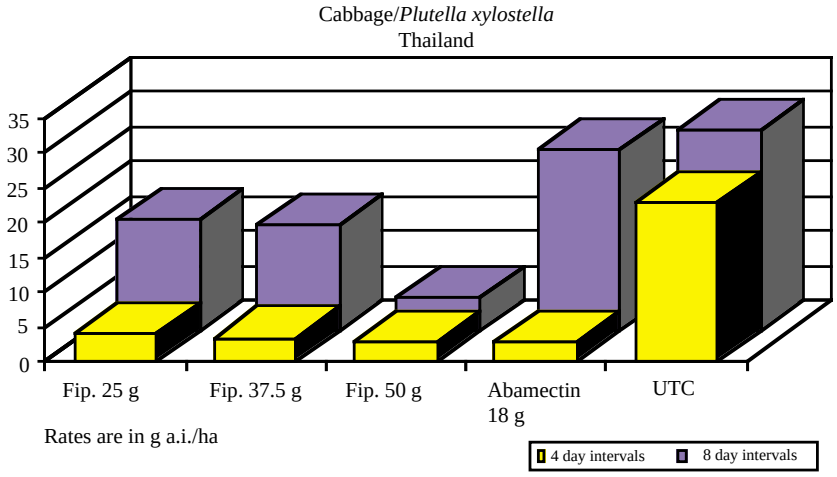


Figure 4. Efficacy of fipronil against Plutella xylostella on cabbage, Thailand.

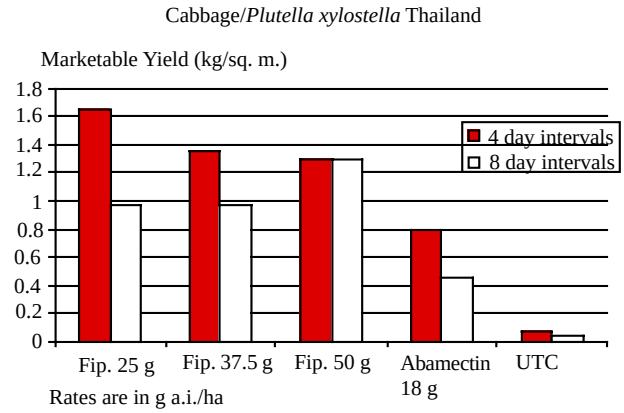


Figure 5. Efficacy of fipronil against Plutella xylostella on cabbage - yields, Thailand.

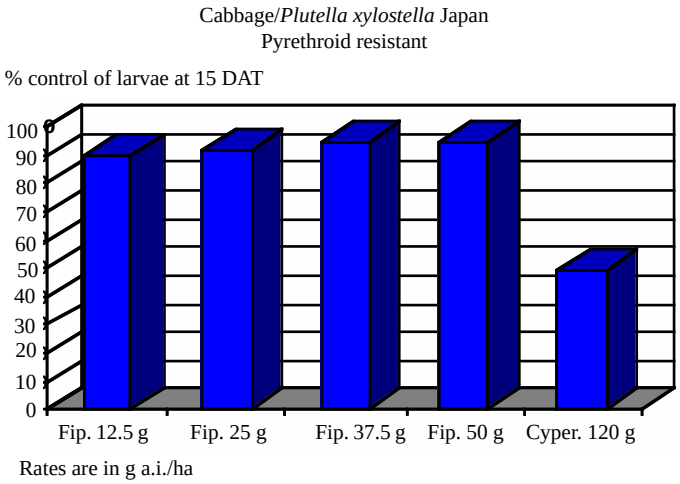


Figure 6. Efficacy of fipronil against pyrethroid-resistant Plutella xylostella on cabbage, Japan.

and improvement in marketable yield) has been superior or equal to that of the standards in 97% of the trials. In 72% of the trials, the performance of fipronil has been superior to that of the standards.

With respect to the results of the resistance monitoring program, in the 18 months that this program has been underway, there have been no changes observed in the susceptibility of DBM to fipronil (at any location in any country). From the beginning of the program, there have been differences noted across different DBM populations relative to the concentration of fipronil necessary to achieve control. In cases where the DBM population in a specific location is well known to possess high levels of resistance to conventional insecticides, the LC_{50} for fipronil can be higher than that for a susceptible population. For example, the LC_{50} for a known susceptible population is 0.082 ppm while the LC_{50} for a field population coming from a resistance area might be 1.6 ppm. The continued monitoring of these populations, however, has demonstrated that there has been no change in the LC_{50}'s over time.

While no indications of resistance development to fipronil have been detected in DBM to date, Rhône-Poulenc is committed to using this resistance monitoring program as a part of its overall strategy for carefully managing the use of fipronil with the objective of ensuring the product remains an effective tool for the control of this important and damaging pest in the coming years.

Involvement of acetylcholinesterase in malathion-resistance of the diamondback moth

Maa, Can Jen William[1], Jin-Fen Liu[1], Yi-Yiu Tzau[1], Ch'ang-Hang Ch'eng[2]
[1]*The Institute of Zoology, Academia Sinica, Nankang, Taipei, Taiwan, R.O.C.*
[2]*Department of Biology, Kao Hsiung Medical School, Kao-Hsiung, Taiwan, R.O.C.*

Abstract

A malathion-resistant strain of diamondback moth, *Plutella xylostella* L., coded MS10 was treated with 250 μg malathion per larva for one generation. Descendants of the treated individuals were mated by method of single female-male pairing. The offsprings were assayed for activities of glutathion-S-transferase, monooxygenase, general esterase, and acetylcholinesterase. The result showed that monooxygenase and acetylcholinesterase were positively involved in malathion resistance of the MS10 diamondback moth. Correlation between the enhanced head acetylcholinesterase and the eclosion rate of the treated larva was statistically significant at a level of $P<0.02$. Correlation between the activity of monooxygenase and the eclosing rate, however, was not significant ($P<0.1$) indicating that the enhanced head acetylcholinesterase was a basic factor for malathion-resistance in the MS substrains. The method of resistance detection mentioned above were used to grade malathion-resistance of 19 local populations of DBM around Taiwan. The results demonstrated that the tolerance of acetylcholinesterase to eserine in these adult insects were statistically correlated with malathion-resistance of these larvae of the wild populations ($P<0.02$). It hinted that an altered acetylcholinesterase coded by a dominant gene can be used as a tool for rapidly evaluating the insecticide resistance of the DBM.

Key words: Acetylcholinesterase, quantitative grading, diamondback moth, resistance

Introduction

Malathion resistance of the diamondback moth (DBM) was suggested to be associated either with enhanced esterase activity (Doichuanngam and Thornhill, 1989) or with monooxygenase activity (Yu and Ngugen, 1992). Wu (1983) indicated that target (acetylcholinesterase) insensitivity of the diamondback moth to organophosphorous insecticides (OP) was also partially involved in resistant mechanism. Sun (1992) indicated that OP resistance seemed to be mediated in part by metabolic and non-metabolic mechanisms. *In vivo* study on degradation of C^{14}-malathion by the Sheh-Tzu (ST) diamondback moth larva revealed that possibly all the metabolic enzyme systems might be associated with the resistance (Maa and Guh, 1988).

The resistant strain, ST10, of DBM is 20-fold resistant to malathion than the susceptible strain, ST12, when LD50 was counted 48hr after treatment. It is a difference of 900-fold between ST10 and ST12 when the eclosion rate of the treated larva was counted. ST10 was characterized as to be with higher frequencies of Est 8b/9b. The ST12 is with higher frequencies of Est 9a/8n (Maa *et al.,* 1992).

Target insensitivity of the resistant strain, ST10, to malathion or its metabolite was also assumed to be associated with the resistance mechanisms of the ST population since we found that the higher frequency of Est 8b was bestowed with the resistant strains and this Est 8b was rarely found in the susceptible strain. Previous study showed that Est 8b was found only in the homogenates of the mid-gut, fat body and central nervous system (Maa *et al.,* 1990). The aberrative growth of the malathion-treated larvae of the ST12 strain was possibly associated with the absence of Est 8b (Maa *et al.,* 1992). Whether the expression of Est 8b in the resistant strain was associated with the 900 fold malathion resistance of ST10 larvae over ST12 larvae was testified in this study.

Intrabreeding of ST strains or substrains has been carried out. Reciprocal crosses were also made between the parent stocks of different strains, substrains. Meanwhile, susceptibility test were carried on, esterase zymogram of all these organisms were investigated, the activity of the degradative enzyme systems as well as the activity of the target enzymes, acetylcholinesterase were also assayed in attempt to explore the following questions: 1. How would Est 8b/9b be involved in malathion-resistance of the DBM? 2. In case the answer to question 1 is yes, would there be any genetic linkage between Est 8b/9b and the resistant mechanism? 3. Which resistant mechanism(s) can be used as parameter(s) to monitor the resistance of the DBM? 4. How would the variation of any resistance mechanism be exploited to discriminate the resistant one from the susceptible one?

Materials and Methods

Insect

Diamondback moth collected from vegetable farms at Sheh-Tzu (ST), Taipei city and other 18 vegetable farms around Taiwan were reared according to Koshihara and Yamada (1981). The 84 hr-old four instar larvae were used for susceptibility test, enzymatic study and the zymogram study as well. The head of 3-day-old adult was used for assay of acetylcholinesterase. Malathion was used only for

discriminating the resistant strain from the susceptible strain and was only selectively applied to DBM as a selection pressure.

Chemicals

All of the chemicals and reagents are of analytical grade or the reagent grade. Diazoblue, alpha-naphthylacetate (1-NA), lauryl sulfate, Fast blue RR for esterase assay; 1,2-dichloro-4-nitrobenzene (DCNB), 1-chloro-2,4-dinitrobezene (CDNB), reduced form of glutathion for glutathion-S-transferase assay; acetylthiocholine iodide for acetylcholinesterase assay were purchased from Sigma Chem. CO., MO, USA. Glucose-6-phosphate dehydrogenase (G-6-P-DH) and other reagents for monooxygenase assay were purchased from Boehringer Mannheim Gmbh Biochemica, Mannheim, Gemany. All of the chemical reagents for electrophoresis were purchased from Bio-Rad., Ca, USA. Paraoxon, o,o-diethyl-o-p-nitro-phenylphosphate; malathion, o,o-dimethyl-s-(1,2-sicarboethoxyethyl) phosphorodithioate, and eserine, were purchased from Chem. Service Co., PA, USA. Malaoxon, o,o-dimethyl-s-(1,2-dicarboethoxyethyl) phosphorothiolate was a gift from Taiwan Branch of American Cyanamid Company.

Strain-selection

The selection was initiated with malathion treatment to the fourth instar larva. The original susceptible strain (SS) was obtained by harvesting the eclosed adults with which their sister-immatures were discriminated by the lowest lethal dose of malathion, and with which their sister larvae were bestowed with the highest frequency of Est 9a in zymogram. In the course of isolation and selection of the susceptible strain single female-male pairing method was followed. The resistant strains were obtained by two ways depending on the experiments: 1. Single female-male pairing method for keeping a strain (RR) with highest LD_{50} against malathion and with highest frequency of Est 8b/9b in the zymogram. 2. Intensively screening of the RR larva with 250μg malathion per larva in order to harvest the individuals and to mate them in group to gain the malathion-selected strain (MS). Selection was orientated to have selection of strain with high fertility for enough offsprings to carry out all the assays. Correlation between the frequency of the Est 8 and mortality rate or eclosion rate of the treated larvae were analyzed to linear regression. Nineteen DBM populations were collected and reared for susceptibility test, enzyme assays and zymogram studies.

Bioassays

Groups of 2-day-old fourth instar larvae were collected from the culture and treated on the dorsal prothorax with defined doses of malathion per larva in 0.2 μl acetone. All tests were replicated twice with 10 larvae per assay. Mortality were counted 48 hr after the treatment and the eclosion rate were also counted accordingly. Dosage-mortality curves were calculated using probit analysis proposed by Finney (1971). The discriminating doses for susceptibility test to the 19 wild populations (see *Figure 1*) were also calibrated by using Abbot's formula.

Enzyme Assays

When microsomal oxidase activity was determined, groups of 20 midgut or carcass of mid body section were dissected from the larvae, and their gut contents or the adhesive tissues or organs were removed. The midguts and the carcass were then washed in 1.15% KCl and homogenized in 0.5 ml ice-cold 0.05M Tris-HCl buffer (0.25% sucrose, 1mM EDTA, 30% glycerol) pH7.5, in a motor-driven grinder for 30 sec. The crude homogenate was centrifuged at 14,000g for 30 min. in an ultracentrifuge. The pellet, which contain cell debris, nuclei, and mitochondria, was discarded. The 14,000g supernatant was used as enzyme source for O-demethylation assay. Para-nitroanisole (PNA) was used as substrate according to Hansen and Hodgson (1971). For glutathion-S-transferase assays, 10 larvae was used for each assay. The 14 000g supernatant of body fraction was prepared with 0.1M reduced glutathion in 0.5ml of 0.1M, pH8.0 Tris-HCl buffer. For DCNB or CDNB conjugation, 80 μl or 20 μl of enzyme solution was added to 800 μl or 860 μl Tris-HCl buffer, mixed with 20 μl DCNB or CDNB and incubated for 5 min. The reaction mixture was then measured at OD344 or OD340 by a spectophotometer, according to Motoyama *et al.*, (1978). Activity of these enzymes were calibrated with extinction coefficient accordingly.

For acetylcholinesterase assay 10 heads of 3-day-old adult were frozen in liquid nitrogen for a couple of days. These pretreated heads were then homogenated in 1.0 to 2.0 ml Tris-HCl buffer (0.05 M, pH7.0, 1% Triton x-100) for 30s and then centrifuged to 10,000 g for 25 min. The supernatant was then used for assay according to Tripathi and O'Brien (1973). Absorption of the product was measured at OD410 nm as described in Ellman *et al.*, (1961). In case the inhibition assay was carried out,

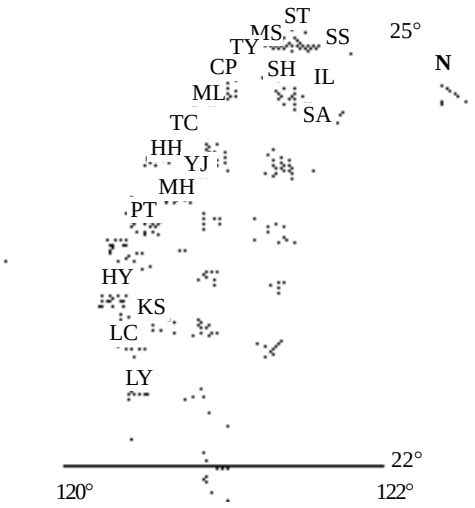


Figure 1. Geographical distribution of the 19 DBM populations in Taiwan

10^{-3} to 5.5 x 10^{-5} M eserine or 10^{-4} to 10^{-6} M paraoxon was preincubated with the enzyme according to Aldridge (1950), Bigley and Plapp (1960). For a routine check up 10^{-4} M of eserine or 10^{-5} M of paraoxon were used.

Data Analysis

Correlation between the frequency of Est 8 of the larvae and 1. Mortality rate of the treated larvae or 2. Eclosion rate of the adult were assayed, respectively by linear regression. Correlation among the discriminating dose of malathion against the larvae of different strains and the activities of various enzymes systems were also analyzed. The same method was applied to the 19 DBM population as well. Probit analysis was performed by a computer program (Finney 1971).

Results and Discussion

Preliminary studies revealed that no correlation of linear regression was found between the high frequencies of Est 8b/9b and the high activities of the glutathion-S-transferase or esterase in the MS strain or the substrain. Sun (1992) indicated that malathion was mainly metabolized by carboxyesterase and not by glutathion-S-transferase. We did find that EST 8/9 could not only hydrolyze malathion but also malaoxon and EST 3/4 could split only malathion but not malaoxon (Maa and Guh, 1988). Nevertheless, in this study the correlation was not found between frequencies of EST 8/ 9 and the activity of the general esterase. Correlation of linear regression between high monooxygenase titer and high frequency of EST 9b was barely significant at P=0.1, although the EST 8b/9b of both MS and RR strains were all with highest frequencies (data not showed).

The specific activities of AchE of adult's head of both the MS and the RR strains are with higher titer than that of the SS strain : a ratio of 1.2 of the MS over 1.0 of the SS (see *Table 1* column 3). Column 4 shows that the head AchE activity of the MS strain, preincubated with eserine, was 1.86 fold as that of the SS strain and was 1.34 fold as that of the RR strain. An enhanced tolerance of MS AchE to eserine, however, was not coordinated with any increasing rate of the EST 8b frequency. In other words, the altered AchE found in MS strain was unlikely genetically cross-linked with EST 8b. Although EST 8b was assumed to be associated with the abberative growth of the treated larvae (Maa *et al.*, 1992), EST 8b could not be used as a parameter to justify the malathion-resistance of the ST population.

AchE activities pretreated with eserine seems to be a better parameter to monitor the resistance of the DBM. *Table 2* column 3 shows that significant correlations of linear regression was found between AchE activity and the survival rate of the MS substrain ($P<0.02$), and between AchE activity and eclosion rate ($P<0.02$). Statistical analysis of the data reveals that a better result of linear regression correlation ($P<0.01$) was found between AchE activity preincubated with eserine and the survival rate or eclosion rate (see *Table 2* column 4).

The above mentioned analysis method was applied to the 18 local DBM populations of Taiwan to justify whether this method is available for monitoring the resistance of the wild DBM population. *Table 3* shows that when MS-3 and MS-19, two random samples taken from the previous set of data (see *Table 2*), were not incorporated with the other 18 objects for the correlation analysis we get a linear regression value ranked in between $0.1<P<0.05$. The wild populations were practically not selected with malathion beforehand and the correlation value was insignificant. However, when MS-3 and MS-19 were added to the set of 18 wild populations as a whole (see *Table 3*) the value for the correlation became significant at $P<0.02$. It hinted that either sample size larger than 17 is required for this linear regression analysis, or some other factor(s) in addition to AchE is necessary for biochemical assay in order to get resistant detection improved. AchE activity has already been used for detection of OP resistance in many insect pests (Dary *et al.*, 1991; Raymond *et al.*, 1985) and other invertebrates (Day and Scott, 1990). It reflected that the uninhibited AchE activity in presence of eserine could be a proper biochemical assay for monitoring the resistance of the DBM either in the laboratory or in the field. Nevertheless, additional improvement for resistance detection based on AchE assay is surely needed.

Acknowledgements

The authors are grateful to the National Science Council, ROC, for financially supporting this research in grants no : NSC81-0211-B001-41 & NSC81-0148-B001-04.

Table 1. Comparison of AchE activity and AchE activity preincubated with eserine in three diamondback moth strains

Strain	AchE activ. µmole/min 0.4H	protein µmg/0.4 Head	AchE specific activ. µmole/min/mg prot.	AchE activ. prein.with Eserine 1.33 x 10^{-5}M µmole/min/mg prot.	AchE activ. uninhibt. rate %
MS[a]	0.235	0.094	2.50	1.915	69.9
SS[b]	0.236	0.113	2.09	1.027	39.4
RR[c]	0.286	0.128	2.23	1.427	60.1

MS[a] : Malathion-selected strain, 250 µg per larva.
SS[b] : Susceptible strain justified by EST8n/9a in zymogram.
RR[c] : Resistant strain justified by EST8b/9b in zymogram.

Table 2. Linear regression correlation between AchE activity, AchE activity after eserine inhibition and survival rate or eclosion rate of the MS substrains

Strain	Surv. rate[b] %	eclosn. rate %	AchE activ. µmole/min/0.4H	AchE activ[c]. pre.with Eserine 1.3×10^{-4} M µmole/min/0.4H	AchE activ. uninhibt. rate %
MS-1[a]	75.00	50.00	0.248	0.186	75.00
MS-2	75.00	75.00	0.281	0.238	84.60
MS-3	76.93	61.54	0.281	0.233	83.00
MS-4	50.00	50.00	0.254	0.193	76.00
MS-5	0.00	0.00	0.104	0.046	44.50
MS-10	57.14	42.86	0.271	0.203	75.00
MS-12	57.14	42.86	0.164	0.126	77.00
MS-13	75.00	50.00	0.258	0.173	67.00
MS-14	90.00	60.00	0.220	0.198	90.00
MS-15	57.14	57.14	0.211	0.161	76.50
MS-19	50.00	37.50	0.208	0.193	93.00
MS-21	71.43	64.29	0.251	0.226	90.00
MS-22	81.82	40.91	0.204	0.172	84.60
MS-25	57.14	50.00	0.228	0.195	85.70
MS-26	63.64	54.55	0.177	0.153	86.70
MS-27	80.00	80.00	0.187	0.164	87.50

[a]MS strain : ST10 diamondback moth selected by 250 µg malation per larva.
[b]MS substrain : 10 adult heads were used for enzyme assay; 20 larva were used for bioassay.
[c]Significant at $P<0.01$ for surv. rate/AchE activ. with eserine.

Table 3. Linear regression correlation between AchE activity preincubated with eserine and survival rate or eclosion rate of the local populations of DBM around Taiwan[a]

Strain	Surv. rate %	Eclosn. rate %	AchE activ. mole/min/0.4H	AchE activ[d]. pre. with Eserine 7.7×10^{-5}M mole/min/0.4H	AchE activ. uninhibt. rate %
SS[b]	5.96	0.00	0.154	0.051	33.30
IL	22.22	15.38	0.281	0.137	48.60
CP	33.33	24.40	0.348	0.192	55.20
SH	25.64	20.90	0.322	0.185	57.50
SA	38.89	44.44	0.215	0.124	57.70
YJ	15.39	7.69	0.382	0.177	46.30
LC	40.74	48.15	0.181	0.087	47.80
KS	32.26	19.35	0.255	0.132	51.90
LY	-------	-------	0.248	0.115	46.40
HL	79.20	62.50	0.322	0.179	55.60
HY	63.16	36.84	0.241	0.116	48.30
MH	11.76	11.10	0.288	0.147	51.60
PT	20.00	20.00	0.281	0.126	44.70
TY	57.20	28.57	0.107	0.067	62.50
TC	55.56	25.93	0.200	0.100	50.00
HH	23.53	23.77	0.375	0.219	58.50
ML	39.40	18.18	0.248	0.144	58.10
ST	55.56	55.56	0.268	0.156	58.30
MS-3[c]	76.93	61.54	0.281	0.176	62.50
MS-19	50.00	37.50	0.208	0.110	53.00

[a]10 adult heads were used for enzyme assay; 30 larvae were used for bioassay with 120µg per larva.
[b]SS : Abbreviation of the site where the insects were collected.
[c]MS : Random selected substrains.
[d]Significant at $P<0.02$ for surv. rate/AchE activ. with eserine.

References

Aldridge, W. N. (1950). Some properties of specific cholinesterase with particular reference to the mechanism of inhibition by diethyl p-nitrophenyl thiophosphate (E 605) and analogues. *Biochem. J.* ***46:*** 451–460

Bigley, W. S. and Plapp, F. W. Jr. (1960). Cholinesterase and aliesterase activity in organophosphorus-susceptible and -resistant house flies. *Ann. Ent. Soc. Am.* ***53:*** 360–364

Day, K. E., and Scott, I. M. (1990). Use of acetylcholinesterase activity to detect sublethal toxicity in stream invertebrates exposed to low concentrations of organophosphate insecticides. *Aquatic Toxicology.* ***18:*** 101–114

Dary, O., Georghiou, G. P., Parsons, E., and Pasteur, N. (1991). Dot-Blot test for identification of insecticide-resistant acetylcholinesterase in single insects. *J. Econ. Entomol.* ***84(1):*** 28–33

Doichuanngam, K. and Thornhill, R. A. (1989). The role of nonspecific esterase insecticide resistance to malathion in the diamondback moth, *Plutella xylostella L. Comp. Biochem. Physiol.* ***93C(1):*** 81–86

Ellman, G., Courtney, D., Andres, V. and Featherstone, R. M. (1961). A new and rapid calorimetric determination of acetylcholinesterase activity. *Biochem. Phatmacol.* ***7:*** 88–95

Finney, D. (1971). *Probit Analysis.* Cambridge Unversity press, Cambridge, England. 333 pp.

Hansen, E. G., and Hodgson, E. (1971). Biochemical characteristic of insect microsome: N-and O-demethylation. *Biochem. Pharmacol.* ***20:*** 1596–1578

Koshihara, T. and Yamada, H. (1981). A simple mass-rearing technique of the diamondback moth, *Plutella xylostella* L. on germinating rape seeds. Jpn. *Appl. Entomol. Zool.* ***20:*** 110–114. (In Japanese, English summary)

Maa, C. J. W. and Guh, S. H. (1988). Biochemical and toxicological properties of a malathion- resistance and -susceptible strain of diamondback moth (*Plutella xylostella* L.). (Abstract). *Proceedings XVIII Intl. Congr. of Entomol.* Vancouver, B.C., Canada., July 3–9, 1988. pp. 465

Maa, C. J. W., Lee, S., Guh, S. H., Wang, C. M., and Hou, J. W. (1992). *Esterase Zymograms as an assay for detection of resistant populations of diamondback moth.* Diamondback Moth and Other Crucifer Pest. (N. S. Talekar ed.). *Proceedings of the Second International Workshop,* Tainan, Taiwan, 10–14 December 1990. Chapter 42, Asian Vegetable Reserch and Development Center, Shanhua, Tainan. pp. 369–381

Maa, C. J. W., Tseng,W. P. and Hung, I. C. (1990). Preliminary characterization of the larva esterases ahd the isozymes of diamondback moth, *Plutella xylostella* (L.). *Bull. Inst. Zool., Academia Sinica.* ***29(3):*** 181–194

Motoyama, N., Kulkarsi, A. P., Hodgson, E., and Dauterman, W. C. (1978). Endogenous inhibitors of glutathion-S-transferase in houseflies. *Pestic. Biochem. Physiol.* ***9:*** 255–262

Raymond, M., Fournier, D., Berge, J., Cuany, A., Bride, J. M., Pasteur, N. (1985). Single- mosquito test to determine genotypes with an acetylcholinesterase insensitive to inhibition to propoxur insecticide. *J. Am. Mosq. Control Assoc.* ***1:*** 425–427

Sun, C. N. (1992). Insecticide resistance in diamondback moth. *In: Diamondback Moth and Other Crucifer Pests.* (N. S. Talekar ed.), *Proceedings of the Second International Workshop,* Tainan, Taiwan, 10–14 December 1990. Asian Vegetable Research and Development Center, Shanhua, Taiwan. pp. 419–426

Tripathi, P.K., and O'Brien, R. D. (1973). Insensitivity of acetylcholinesterase as a factor in resistance of houseflies to the organophosphate Rabon. *Pest. Biochem. Physiol.* ***3:*** 492–498

Wu Tzw-Kann (1983) Relationship between resistance to organophosphorous and carbamate insecticides and acetylcholinesterase in the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Yponomeutidae). Masters thesis, Research Institute of Entomology, National Chung-Hssing University, pp. 47

Yu, S. J. and Ngugen, S. N. (1992). Detection and biochemical characterization of insecticide resistance in the diamondback moth. *Pestic. Biochem. Physiol.* ***44:*** 74–81.

Resistance mechanisms to *Bacillus thuringiensis* subspp. *kurstaki* and *aizawai* in a multi-resistant field population of *Plutella xylostella* from Malaysia

Denis J. Wright[1], Muhammad Iqbal[1], Francisco Granero[2] and Juan Ferre[2]

[1]*Biology Department, Imperial College at Silwood Park, Ascot, Berkshire SL5 7PY, UK;*
[2]*Departamento de Genética, Universitat da Valencia, 46100 Burjassot, Valéncia, Spain,*

Abstract

A field population (SERD3) of *Plutella xylostella* L. collected in December 1994, and resistant to various insecticides, including *Bacillus thuringiensis* subsp. *kurstaki* (*Btk*) and *B.t.* subsp. *aizawai* (*Bta*), was selected with *Btk* (*Btk*-Sel) and *Bta* (*Bta*-Sel). Little evidence of cross-resistance was observed. Binding to midgut brush border membrane vesicles was examined for insecticidal crystal proteins specific to *Btk* [Cry1Ac], *Bta* [Cry1Ca] or to both [Cry1Aa and Cry1Ab]. In SERD3 (c. 50 and 30-fold resistance to *Btk* and *Bta*), specific binding of Cry1Aa, Cry1Ac and Cry1Ca was similar compared with a susceptible population (ROTH) but binding of Cry1Ab was minimal. The *Btk*-Sel (c. 600 and 60-fold resistance to *Btk* and *Bta*) and *Bta-Sel* (c. 80 and 300-fold resistance to *Btk* and *Bta*) populations lacked binding to Cry1Ab but in *Bta*-Sel binding of Cry1Ca was similar to ROTH. The results suggest reduced binding of Cry1Ab can partly explain resistance to *Btk* and *Bta*. However, the binding of Cry1Aa, Cry1Ac and Cry1Ca in resistant populations, and the lack of cross-resistance between *Btk* and *Bta*, also suggests additional resistance mechanisms are present.

Key words: *Bacillus thuringiensis*: *Plutella xylostella*; resistance; mechanisms.

Introduction

After several decades of usage of products based on insecticidal crystal proteins (ICPs) of *Bacillus thuringiensis (B.t.)*, the 1990s saw an increasing number of reports of field resistance (Tabashnik, 1994), although this has only been fully substantiated in *Plutella xylostella* L. (Lepidoptera: Yponomeutidae). Field resistance in this species can be correlated with increasing usage of *B.t.* products during the 1980s (Tabashnik, 1994). Similar increases in selection pressure are likely to occur for other species with the introduction of new *B.t.* strains and transgenic crops expressing genes encoding for ICPs (e.g. Tabashnik, 1994). Characterising resistance to ICPs is thus an important component of strategies aimed at safeguarding their future use (Ferré *et al*., 1995).

In a laboratory-selected population of the Indian meal moth, *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae), resistance to Dipel^{R} *(B.t.* subsp. *kurstaki)* and a constituent ICP, Cry1Ab, was correlated with reduced binding of the latter to a midgut membrane receptor (Van Rie *et al*., 1990a). Studies with *P. xylostella* from the Philippines, Hawaii and Florida also demonstrated reduced binding of Cry1Ab or Cry1Ac to midgut membrane receptors (Tabashnik *et al*., 1994; Ferré *et al*., 1995; Tang *et al*., 1996). However, there have been cases of laboratory-selected insects for which non receptor-related resistance mechanisms have been proposed (see Tabashnik, 1994; Moar *et al*., 1995; Forcada *et al*., 1996).

Products based on *B.t.* subsp. *aizawai* have been introduced relatively recently and while a few cases of low level resistance have been reported in field populations of *P. xylostella*, these have generally been attributed to cross-resistance with *B.t. kurstaki* (Tabashnik, 1994; Iqbal *et al*., 1996). The present paper reports studies on resistance mechanisms to *B.t. kurstaki* and *B.t. aizawai* in a field population of *P. xylostella* from Malaysia with relatively high levels of resistance to both *B.t.* subspp.

Materials and methods.

Bacillus thuringiensis products.

Test solutions were freshly prepared from Dipel^{R} (*B.t. kurstaki* strain HD-1, containing ICPs - Cry1Aa, Cry1Ab, Cry1Ac, Cry2Aa & Cry2Ab; 16000 International Units (IU) mg^{-1} wettable powder; Abbott Laboratories) and Florbac^{R} (*B.t. aizawai*, containing Cry1Aa, Cry1Ab, Cry1Ca & Cry1Da; 8500 IU mg^{-1} flowable concentrate; Novo Nordisk) in distilled water with Triton X-100 (50 µg ml^{-1}) as a surfactant.

Insects

An insecticide susceptible, laboratory strain of *P. xylostella* (ROTH) was obtained from IACR Rothamsted (Harpenden, UK). A field population (SERD3) was collected from crucifer crops in Serdang, Selangor, Malaysia in December 1994 and imported into the UK at the F6 generation (c. 200 insects). Insect larvae were reared and tested on organically-grown 4–6 week-old Chinese cabbage, *Brassica chinensis* var. *pekinensis* cv. Tip Top, at 20 °C, c. 65% r.h. and 16 h

photophase (Iqbal *et al*., 1996). The SERD3 population was divided into three sub-populations at F7. Two were selected with *B.t. kurstaki* (*Btk*-Sel) and *B.t. aizawai* (*Bta*-Sel) from F7 to F9 (mean survival to adult = 58 and 46% for *Btk* and *Bta* respectively), the third was left unselected (SERD3).

Toxicity bioassays

Third instar larvae (at F7 and F10) were tested using a leaf-dip bioassay (Iqbal *et al*., 1996). Each leaf disc (4.8 cm dia.) was immersed in test solution for 10 s. Controls were immersed in distilled water with Triton X-100. Five larvae were placed on each leaf disc and each treatment was replicated 10 times. After 5 days, the remains of the leaf discs were removed and replaced by fresh, untreated leaves. Mortality was assessed after 9 days (Iqbal *et al*., 1996). Where necessary, bioassay data were corrected for control mortality (Abbott, 1925). Estimates of LC_{50} values and their 95% fiducial limits (FL) were obtained by maximum likelihood logit regression analysis in GLIM 3.77 (Numerical Algorithms Group, Oxford, 1985) using generalised linear modelling techniques from which differences between data sets were extracted by analysis of deviance (Crawley, 1993). LC_{50} values were compared using individual 95% FL for two parameters (p = 0.01). An estimate of 'realised heritability' (h^2), the proportion of phenotypic variance accounted for by additive genetic variation, was calculated (Tabashnik, 1992) to compare the rate of selection in different populations.

Bacillus thuringiensis ICPs and iodination procedure

Trypsin-activated Cry1Aa, Cry1Ab and Cry1Ac were kindly provided by Luke Masson (National Research Council of Canada, Montreal) and Cry1Ca by Jeroen Van Rie (Plant Genetic Systems, Gent, Belgium). Cry1A ICPs were obtained as recombinant proteins, cloned from the NRD-12 strain of *B.t. kurstaki*, and expressed in *Escherichia coli* (Masson *et al*., 1989). Cry1Ca was a recombinant protein, cloned from *B. thuringiensis* var. *entomocidus* HD-110, and expressed in *E. coli* (Ferré *et al.*, 1991). The protein concentration of purified trypsin-activated toxins was determined using the method of Bradford (1976) with bovine serum albumin (BSA) as standard. Iodination of Cry1A ICPs was carried out by the chloramine-T method (Van Rie *et al*., 1990b). Cry1Ca toxin was labelled by means of the Iodogen method (Hoffman *et al*., 1988).

Preparation of brush border membrane vesicles and binding assays

Brush border membrane vesicles (BBMV) from each population of *P. xylostella* were prepared (Escriche *et al.*, 1995) from whole last instar larvae (c. 1 500 larvae from each population), frozen in liquid nitrogen and stored at -80°C. Protein concentrations in the BBMV preparations were determined by the method of Bradford (1976). The conditions used for the binding assays were essentially the same as previously published (Ferré *et al*., 1995). Duplicate samples of BBMVs and labelled ICPs were incubated in PBS/ 0.1% BSA (0.1 ml final volume) at room temperature. After 30 min (for Cry1A's) or 90 min. (for Cry1Ca), the reaction was stopped by filtration through Whatman GF/F glass-fibre filters (previously soaked with PBS/0.5% BSA) in a Millipore manifold sample filtration unit. Filters were washed rapidly with 5 ml of ice-cold PBS/0.1% BSA and transferred to microtubes. The radioactivity retained in the filters was measured in a 1282 Compugamma CS gamma-counter (LKB). Non-specific binding was determined by adding a 100-fold excess of the corresponding unlabelled ICP. For competition experiments, 9–10 µg of BBMV proteins were incubated with labelled ICPs at increasing concentrations of the corresponding unlabelled ICP. Quantitative binding data were obtained from competition experiments using the LIGAND computer programme (Ferré *et al.*, 1991).

Results

Toxicity of B. t. products

The F7 generation of the SERD3 population was c. 330-fold resistant to *B.t. kurstaki* and 160-fold resistant to *B.t. aizawai* (at LC_{50} level) compared with the control, ROTH population *(Table 1)*. Selection of sub-populations of SERD3 with *B.t. kurstaki* and *B.t. aizawai* increased resistance c. 2-fold in the F10 generation compared with the F7 generation while resistance to these *B.t.* products declined c. 7- and 5-fold respectively in the unselected sub-population of SERD3. The slope of the regression for *B.t. kurstaki* against the F7 generation of SERD3 was markedly greater ($P<0.05$) compared with the F10 generations of SERD3 and *Btk*-Sel *(Table 1)*.

In the laboratory generation (F10) of SERD3 examined in subsequent binding assays, the level of resistance to *B.t. kurstaki* (c. 600) and *B.t. aizawai* (c. 300) was thus an order of magnitude greater in both the *Btk*-Sel and *Bta*-Sel sub-populations respectively compared with the concurrent generation (F10) of the unselected SERD3 sub-population *(Table 1)*. The corresponding level of cross-resistance to *B.t. aizawai* and *B.t. kurstaki* in *Btk*-Sel and *Bta*-Sel respectively was < 2-fold greater ($P>0.01$) compared with unselected SERD3 at F10. Estimations of realised heritability *(h^2)* of resistance gave an intermediate value for *B.t. kurstaki* and a relatively low value for *B.t. aizawai (Table 1)*.

Binding of iodinated ICPs to BBMVs.

BBMV from unselected (SERD3) and insecticide susceptible, control (ROTH) insects were tested for binding with ^{125}I-labelled. Saturable specific binding was found for all ICPs tested with BBMV from ROTH. In contrast, BBMV from SERD3 showed strongly reduced binding of ^{125}I-labelled Cry1Ab (maximum binding was 0.6 % compared with 3.8 % for ROTH). Saturation experiments with SERD3 and ROTH did not appear to differ appreciably for the other three

Table 1. Toxicity of *B.t.* subsp. *kurstaki* (*Btk*) and *B.t.* subsp. *aizawai* (*Bta*) against a *B.t.* susceptible (ROTH) and a field population (SERD3) of *Plutella xylostella*, and to laboratory-selected sub-populations of SERD3 (*Btk*-Sel and *Bta*-Sel)[1,2]

Population (generation)	Test product	LC_{50} (95% FL)[3]	SLOPE ± SE	Resistance ratio[4]
ROTH	*Btk*	0.018 (0.013–0.022)	2.92 ± 0.46	–
SERD3 (F7)	*Btk*	5.89 (4.66–7.60)	3.85 ± 0.52	330
SERD3 (F10)	*Btk*	0.87 (0.64–1.24)	2.16 ± 0.25	50
Btk-Sel (F10)	*Btk*	10.7 (7.65–15.9)	2.00 ± 0.25	600 (13)
Bta-Sel (F10)	*Btk*	1.40 (1.01–2.08)	2.10 ± 0.24	80 (2)
ROTH	*Bta*	0.012 (0.009–0.017)	2.20 ± 0.24	–
SERD3 (F7)	*Bta*	1.92 (1.33–2.93)	1.77 ± 0.21	160
SERD3 (F10)	*Bta*	0.38 (0.24–0.59)	1.38 ± 0.18	30
Btk-Sel (F10)	*Bta*	0.67 (0.49–0.91)	2.42 ± 0.29	60 (2)
Bta-Sel (F10)	*Bta*	3.64 (2.74–5.01)	2.33 ± 0.21	300 (10)

[1]Leaf-dip assay against third instar larvae; mortality assessed after 9 days.
Sub-populations selected from F7 to F9.
[2]Estimates of 'realized heritability' (h^2) (Tabashnik, 1992) for *Btk*-Sel and *Bta*-Sel over three generations = 0.441 and 0.243 respectively.
[3]Units: IU/mg.
[4]Resistance ratio compared with equivalent LC_{50} value for ROTH population.
Values in parentheses = resistance ratio compared with unselected SERD3 at F10.

Table 2. Equilibrium dissociation constants (K_d) and concentration of receptors (R_t) of *B.t.* ICPs on BBMV for a control (ROTH) and two resistant (unselected SERD3 and *Bta*-Sel) populations of *P. xylostella*[1]

ICP	Population	K_d, nM	R_t, pmol/mg BBMV protein
Cry1Ac	ROTH	22.4 (0.1)	2.7 (0.1)
	SERD3 (F10)	27.3 (0.3)	3.3 (0.1)
Cry1Ca	ROTH	8.9 (0.1)	9.2 (1.0)
	Bta-Sel (F10)	8.7 (0.1)	9.0 (0.9)

[1]K_d and R_t values (mean ±SE) were calculated from homologous competition experiments for Cry1Ac (n = 2) and Cry1Ca (n = 2) performed on the same batch of BBMV.

ICPs. Maximum specific binding was 4.6%, 1.7% and 5.4% for SERD3 and 3.6%, 1.7% and 6.9% for ROTH, with Cry1Aa, Cry1Ac and Cry1Ca respectively.

Because of the low specific binding obtained with Cry1Ac, competition experiments were conducted to confirm the specificity of binding and so discard the possibility of an artifact in the saturation experiment. Results showed *(Table 2)* that binding of ^{125}I-labelled Cry1Ac was competed by non-labelled Cry1Ac, giving typical curves for this type of experiment. Neither the equilibrium dissociation constant (K_d) nor the receptor concentration (R_t) were noticeably different for Cry1Ac between the two insect populations *(Table 2)*.

Analysis of BBMV from the two selected resistant populations (*Btk*-Sel and *Bta*-Sel) with ^{125}I-labelled Cry1Ab also showed strongly reduced binding to this ICP (0.6 % and 0.3 % respectively). Unfortunately, it was not possible to conduct binding assays for these populations with either Cry1Aa or Cry1Ac.

Since Cry1Ca is a component of Florbac^R but is not present in Dipel^R, binding experiments were conducted with BBMV from the *Bta*-Sel population to test whether selection had changed the affinity for this ICP. No reduction in specific binding of Cry1Ca was detected in saturation experiments (maximum binding = 8.8 %). Moreover, competition experiments with *Bta*-Sel and ROTH did not show any noticeable differences in binding affinity or receptor concentration *(Table 2)*.

Discussion

In contrast to previous field populations of *P. xylostella* examined (e.g. Tabashnik, 1994; Tang *et al.*, 1996; Iqbal *et al.*, 1996), SERD3 appeared to show appreciable levels of resistance to *B. t. aizawai* in addition to resistance to *B. t. kurstaki*. The selection experiments confirmed resistance to both *B.t.* subspp. and showed that selection of resistance occurred at a greater rate to *B.t. kurstaki* compared with *B.t. aizawai* under our laboratory conditions. The lack of cross-resistance in the *Btk*-Sel and *Bta*-Sel populations suggests the presence of gene(s) for resistance to toxic components in *B.t. kurstaki* and *B.t. aizawai* which segregate independently. Liu *et al*. (1996) have recently provided evidence that gene(s) conferring resistance to Cry1Ab and Cry1Ca segregate independently in a field population of *P. xylostella* from Hawaii.

The binding experiments appear to indicate that the only major biochemical difference between the ROTH and SERD3 populations of *P. xylostella* is reduced binding of the latter to Cry1Ab. However, it is noteworthy that binding to Cry1Aa and Cry1Ac was not affected, despite their close structural relationship with Cry1Ab. Furthemore, it seems to be a common feature that these three ICPs share common binding sites in many lepidopteran species, including *P. xylostella* (Escriche *et al.*, 1996). This feature has helped promote the assumption that insects resistant to commercial products of *B.t. kurstaki*, for which

reduced binding to Cry1Ab or Cry1Ac has been shown, also have reduced binding to other Cry1A ICPs.

One of the first and most widely used *B.t. kurstaki* products, Dipel^R, is based on strain HD-1 which produces Cry1Aa, Cry1Ab, Cry1Ac, Cry2Aa and Cry2Ab toxins. However, Cry2Aa is reported to be scarcely toxic to *P. xylostella* (Tang *et al.*, 1996) and this also likely to be the case for Cry2Ab. Therefore, a change in a common receptor for the three Cry1A ICPs would probably be sufficient to confer to *P. xylostella* resistance to *B. t. kurstaki* HD-1 products. This is clearly not the case in the SERD3 population of *P. xylostella*. Thus, despite Cry1A ICPs sharing common binding sites, Cry1Ab at least must have another, independent binding site(s) susceptible to change without affecting binding to the other ICPs. Lack of cross-resistance to other Cry1A toxins in a population of *P. xylostella* from the Philippines highly resistant to Cry1Ab also suggests the presence of independent binding sites (Ballester *et al.*, 1994) although in the latter there was no resistance to *B.t. kurstaki*.

How can reduced binding of Cry1Ab confer resistance to *B.t. kurstaki* and *B.t aizawai* products? The composition of ICPs in a Dipel^R formulation has been reported to be 28% Cry1Aa, 53% Cry1Ab, 19% Cry1Ac, while another *B.t. aizawa*i product, XenTari^R, contains 32% Cry1Aa, 38% Cry1Ab, 26% Cry1Ca and 5% Cry1Da (Liu *et al*., 1996). The toxicities of these ICPs in a susceptible colony of *P. xylostella* (Tang *et al*., 1996) were found to be 0.3 for Cry1Aa, 0.6 for Cry1Ab, 1.1 for Cry1Ac, 4.3 for Cry1Ca and 0.2 for Cry1Da (LC_{50} in µl/ml). If both parameters are combined, assuming similar joint action of the components (Tang *et al.*, 1996), the contribution of Cry1Ab to the toxicity of both formulations is considerable (c. 40% and 30% for Cry toxins in Dipel^R and XenTari^R respectively). Thus, reduced binding of CryIAb alone could confer some degree of resistance to both *B.t. kurstaki* and *aizawai* formulations. A similar calculation would suggest that Cry1Ca contributes relatively little to the toxicity of, and thus resistance to, *B.t. aizawai* (XenTari^R), an observation supported by the much greater degree of resistance observed to Cry1Ca compared with *B. t. aizawai* in a field population of *P. xylostella* from Hawaii (Liu *et al*., 1996).

However, while a modification in the binding site, specific for Cry1Ab, can explain increased resistance to *B.t. kurstaki* and *aizawai* formulations, it cannot explain the differences in susceptibility of the three resistant populations (SERD3, *Btk*-Sel and *Bta*-Sel) after selection in the laboratory for three generations. Our results show that selection with *B.t. kurstaki* did not noticeably decrease binding of Cry1Ab, although this was minimal anyway in SERD3 at F10, nor selection with *B.t. aizawai* change binding parameters for Cry1Ca. A possible explanation, although unlikely, is that selection acted upon binding affinities of ICPs not tested with the selected populations (Cry1Aa, Cry1Ac and Cry1Da). Alternatively, SERD3 had other, independent mechanisms of resistance that potentiated the effect of the modified receptor for Cry1Ab. If the latter were true, resistance due to these non-receptor related mechanisms seems to have been unstable, and only selection with the other *B.t*. formulations maintained or increased them.

Interestingly, studies on *P. interpunctella* have shown that laboratory-selection with *B.t. kurstaki* causes relatively narrow-spectrum resistance to Cry1Ab and Cry1Ac, while selection with *B.t. aizawai* causes broader-spectrum resistance to Cry1A ICPs, Cry1B, Cry1C and Cry2A ICPs (McGaughey & Johnson, 1994). The presence of independent, unstable resistance mechanisms could account for the apparent lack of cross-resistance observed in the bioassays with the *Btk*-Sel and *Bta*-Sel populations. Such mechanisms may be related to any other step in the mode of action of ICPs (Knowles, 1994).

Finally, we must not forget another component of *B. thuringiensis* formulations: the spore. Besides its intrinsic toxic effect, synergism between spores and ICPs has been reported recently in *P. xylostella* (Tang *et al*., 1996) and the beet armyworm, *Spodoptera exigua* Hübner (Lepidoptera; Noctuidae) (Moar *et al.*, 1995), although its synergistic effect with *B. thuringiensis* crystals is long known (Li *et al*., 1987). It is logical to think that there must be genes controlling sensitivity to the spore and to its synergistic effects. This possibility has just started to be explored (Tang *et al.*, 1996).

Acknowledgments

We thank Dzolkhifli Omar for collecting the insects in Malaysia, and Luke Masson and Jeroen Van Rie for providing ICPs. M.I. was supported by a scholarship from the Government of Pakistan and by an O.R.S. award (UK). This work was conducted under M.A.F.F. licence no.s PHF 909A/1447/45 and PHF 909A/1031/54.

References

Abbott, W. S. (1925). A method for computing the effectiveness of an insecticide. *J. Econ. Entomol.* ***18:*** 265–267.

Ballester, V., Escriche, B., Ménsua, J. L. , Riethmacher, G. W. and Ferré, J. (1994). Lack of cross-resistance to other *Bacillus thuringiensis* crystal proteins in a population of *Plutella xylostella* highly resistant to CryIA(b). *Biocontrol Sci. Technol.* ***4:*** 437–443.

Bradford, M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* ***72:*** 248–254.

Crawley, M. J. (1993). *GLIM for Ecologists*, Blackwell, London, 379 pp.

Escriche, B., Silva, F. J. and Ferré, J. (1995). Testing suitability of brush-border membrane vesicles prepared from whole larvae of small insects for binding studies with *Bacillus thuringiensis* CryIA(b) crystal protein. *J. Invert. Pathol.* ***65:*** 318–320

Escriche, B., Ferré, J.and Silva, F. J. (1996). Personal communication.

Ferré, J., Real, M. D., Van Rie, J., Jansens, S. and Peferoen, M. (1991). Resistance to the *Bacillus thuringiensis* bioinsecticide in a field population of *Plutella xylostella* is due to a change in a membrane midgut receptor. *Proc. Natl. Acad. Sci. USA* ***88:*** 5119–5123.

Ferré, J., Escriche, B., Bel, Y. and Van Rie, J. (1995). Biochemistry and genetics of insect resistance to *Bacillus thuringiensis* insecticidal crystal proteins. *FEMS Microbiol. Lett.* ***132:*** 1–7.

Forcada, C., Alcacer,E., Garcera, M. D. and Martinez, R. (1996). Differences in the midgut proteolytic activity of two *Heliothis virescens* strains, one susceptible and one resistant to *Bacillus thuringiensis* toxins. *Arch. Insect Biochem. Physiol.* ***31:*** 257–272.

Hoffman, C., Luthy, P., Hutter, R. and Pliska, V. (1988). Binding of the delta-endotoxin from *Bacillus thuringiensis* to brush-border membrane vesicles of the cabbage butterfly (*Pieris brassicae*). *Eur. J. Biochem.* ***173:*** 85–91.

Iqbal, M., Verkerk, R. .H. J., Furlong, M. .J., Ong, P-C., Syed, A. R. and Wright, D. J. (1996). Evidence for resistance to *Bacillus thuringiensis* (*Bt*) subsp. *kurstaki* HD-1, *Bt* subsp. *aizawai* and abamectin in field populations of *Plutella xylostella* from Malaysia. *Pestic. Sci.* ***48:*** (in press).

Knowles, B. H. (1994). Mechanism of action of *Bacillus thuringiensis* insecticidal delta-endotoxins. *Adv. Insect Physiol.* ***24:*** 275–308.

Li, R. S., Jarret, P. and Burges, H. D. (1987). Importance of spores, crystals, and delta-endotoxins in the pathogenicity of different varieties of *Bacillus thuringiensis* in *Galleria mellonella* and *Pieris brassicae*. *Invertebr. Pathol.* ***50:*** 277–284.

Liu, Y-B., Tabashnik, B. E. and Pusztai-Carey, M. (1996). Field evolved resistance to CryIC in diamondback moth (Lepidoptera: Plutellidae). *J. Econ. Entomol.* ***89:*** 798–804.

Masson, L., Préfontaine, G., Péloquin, L., Lau, P. C. K. and Brousseau, R. (1989). Comparative analysis of the individual protoxin components in P1 crystals of *Bacillus thuringiensis* subsp. *kurstaki* isolates NRD-12 and HD-1. *Biochem. J.* ***269:*** 507–512.

McGaughey, W. H. and Johnson, D. E. (1994). Influence of crystal protein composition of *Bacillus thuringiensis* strains on cross-resistance in Indian meal moths (Lepidoptera: Pyralidae). *J. Econ. Entomol.* ***87:*** 535–540.

Moar, W. J., Pusztai-Carey, M., Van Faassen, H., Bosch, D., Frutos, R., Rang, C., Luo, K. and Adang, M. J. (1995). Development of *Bacillus thuringiensis* CryIC resistance by *Spodoptera exigua* (Hbner) (Lepidoptera: Noctuidae). *Appl. Environ. Microbiol.* ***61:*** 2086–2092.

Tabashnik, B. E. (1992). Resistance risk assessment: realized heritability of resistance to *Bacillus thuringiensis* in Diamondback Moth (Lepidoptera: Plutellidae), Tobacco Budworm (Lepidoptera: Noctuidae) and Colorado Potato Beetle (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* ***85:*** 1551–1559.

Tabashnik, B. E. (1994). Evolution of resistance to *Bacillus thuringiensis. Annu. Rev. Entomol.* ***39:*** 47–79.

Tabashnik, B. E., Finson, N., Groeters, F. R., Moar, W. J., Johnson, M. W., Luo, Ke and Adang, M. J. (1994). Reversal of resistance to *Bacillus thuringiensis* in *Plutella xylostella*. *Proc. Natl. Acad. Sci. USA* ***91:*** 4120–4124.

Tang, J. D., Shelton, A. M., Van Rie, J., De Roeck, S., Moar, W. J., Roush, R. T. and Peferoen, M. (1996). Toxicity of *Bacillus thuringiensis* spore and crystal protein to resistant diamondback moth (*Plutella xylostella*). *Appl. Environ. Microbiol.* ***62:*** 564–569.

Van Rie, J., Jansens, S., Hofte, H., Degheele, D. and Van Mellaert, H. (1990a). Receptors on the brush border membrane of the insect midgut as determinants of the specificity of *Bacillus thuringiensis* delta-endotoxins. *Appl. Environ. Microbiol.* ***56:*** 1378–1385.

Van Rie, J., McGaughey, W. H., Johnson, D. E., Barnett, B. D. and Van Mellaert, H. (1990b). Mechanism of insect resistance to the microbial insecticide *Bacillus thuringiensis*. *Science* ***247:*** 72–74.

Biochemical and physiological characteristics in chlorfluazuron resistant diamondback moth

Tadashi Miyata, Taslima Rahman, Wu Gang and Adel Ramzy Fahmy

Laboratory of Applied Entomology, Faculty of Agricultural Sciences, Nagoya University, Chikusa, Nagoya, 464-01 Japan

Abstract

Diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) showed a high resistance to chlorfluazuron by laboratory selection. However, this resistance was not stable under the laboratory condition without chlorfluazuron selection. When *in vivo* metabolism of ^{14}C-chlorfluazuron was studied, higher metabolic activities were observed in the chlorfluazuron resistant strain than in the susceptible one. From the metabolites which were obtained by carboxyamide cleavage, it was considered that higher carboxyamidase activity in the resistant diamondback moth was involved as one of important causes of resistance. Assay of carboxyamidase activity *in vitro* showed that the enzyme activity was higher in the resistant strains than in the susceptible ones. The sensitivity of male adults of diamondback moth to synthetic sex pheromone was compared between two strains by a bioassay method in the laboratory. The results indicated that chlorfluazuron resistant strains responded to a wider range of synthetic sex pheromone concentration than that of the susceptible ones. The results obtained here require further studies, however, they showed interesting points to understand the mechanism of chlorfluazuron resistance and control by mating disruption method in the diamondback moth.

Key words: chlorfluazuron, resistance, carboxyamidase, sex pheromone, diamondback moth.

Introduction

Diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) (DBM), is one of the most notorious insects due to its development of insecticide resistance to every insecticide in tropical and temperate areas of the world (Talekar and Griggs, 1986; Talekar, 1992; Saito *et al.,* 1995). Chlorfluazuron is one of benzoylphenyl urea compounds and highly effective for DBM control. In spite of many reports on the development of chlorfluazuron resistance in DBM, studies on mechanisms of chlorfluazuron resistance are very few (Talekar, 1992). Chlorfluazuron resistance is not so stable free from the selection pressure (Fahmy and Miyata, 1992; Sinchaisri *et al.,* 1989).

In this paper, we tried to clarify the biochemical and physiological characteristics in chlorfluazuron resistant DBM so that we can get basic informations to manage the development of chlorfluazuron resistance.

Materials and methods

Insects

Two Thailand strains of DBM, Tup Luang (TL) and Bang Khae (BK) were mainly used. At the time of collection these strains were from 400 to 3400-fold resistant to chlorfluazuron compared to a Japanese Osaka susceptible strain (Sinchaisri *et al.,* 1989). After being reared in the laboratory for two years without any insecticidal pressure, they were imported to Japan. A portion of each strain was selected with chlorfluazuron 25 times among 33 generations in the laboratory (Fahmy, 1993). Then they were occassionally selected with chlorfluazuron to keep high resistance levels. The selected strain was regarded as resistant and non-selected one as susceptible.

For carboxyamidase assay, a susceptible strain, Osaka susceptible strain (Noppun *et al.,* 1983), and two field collected resistant strains were used. These field strains showed high resistance to various types of insecticides including chlorfluazuron.

They were reared at 25°C and photoperiod of 16:8 (L:D). The rearing conditions are the same as Fahmy *et al.,* (1991).

Chemicals

^{14}C-Labeled chlorfluazuron (654 MBq/mmol) which was uniformly labeled at the 2,5-difluorophenyl ring was received from Ishihara Sangyo Kaisha, Ltd., Tokyo. Unlabeled proposed metabolites i.e. 2,6-difluorobenzoic acid and 2,6-difluorobenzamide were bought from Wako Pure Chemical Industries, Ltd., Tokyo, Japan. Triphenyl phosphate (TPP) (95% purity) was used as a synergist. Silica gel 60 F_{254} pre-coated plates (Merck, Darmastadt, Germany) were used for thin layer chromatography (TLC) and Fuji medical X-ray films (Fuji Photo Film Co., Ltd., Japan) were used for tracing radioactivity. Acetyl *p*-nitoranilide was bought from Wako Pure Chemical Industries, Ltd. Synthetic sex pheromone of DBM (Z)-11-hexacenayl acetate, (Z)-11-hexacenal and (Z)-11-hexadecen-1-o1 at 50:50:1(W/W) mixture in hexane solution, was a gift from Takeda Chemical Industries Co. Ltd. (Tokyo, Japan).

In vivo ***metabolism of ^{14}C-chlorfluazuron***

Early third instar larvae were starved for 24 hr before experiment to ensure a whole consumption of the

treated leaf. Two µl acetone solution containing 10^{-3M} of radiolabeled chlorfluazuron, or chlorfluazuron with TPP at 1:5 ratio (W/W) was applied to a small cabbage leaf disc (1mm x 1mm). They were left to air dry and then put singly in a glass homogenizer. After that larvae were introduced singly to each glass homogenizer. For each treatment, five larvae with three replicates were used. The leaf disc was totally consumed by each larva within four hours. The five larvae of each test were then collected in one glass homogenizer and were incubated thereafter for 96 hr at 25 °C. The glass homogenizers were then rinsed three times with 1 ml of extraction solvent (acetonitrile:ethyl acetate:methanol:water, 1:1:1:1. v/v) (El-Saidy *et al.*, 1989) and the rinsing solution was added to the glass homogenizer containing the larvae. The larvae were then homogenized in the same solution for one min. at 4 °C. The products were extracted successive three times by addition of one ml of the extraction solvent then centrifuged at 1500 rpm for 5 min. The combined extracts were concentrated under vacuum at 35 °C using a rotary evaporator. The extract was analyzed by TLC using a developing solvent system (ethyl acetate: toluene:acetic acid, 50:45:5, v/v). The plate was then exposed to X-ray film for one week to trace radioactivity. After that the silica gel plate was cut to small pieces (5 mm width), scratched and introduced to a scintillation glass vial containing 5 ml aqueous counting scintillant, ACS II® (Amersham Corporation, U.S.A.) and radioactivity was measured by a liquid scintillation spectrophotometer (Beckman, Beckman Instruments Nuclear Systems Operations, CA, U.S.A.). Ultraviolet light was used to detect the authentic unlabeled compounds.

In vitro ***assay of carboxyamidase***

Third instar DBM larvae were homogenized in 0.05M, pH 7.5 Tris-HCl buffer. The 1,000 g supernatant of the homogenate was used as an enzyme source. The assay of carboxyamidase with acetyl *p*-nitroanilide as a substrate was performed at 30 °C (Woods *et al.*, 1979).

Observation of male response to synthetic sex pheromone

Males were sexed at the larval stage and reared separately. Two yellowish spots on the 2/3rd segment of the larval body indicate testis of males. To each 200 ml conical flask, 5% honey solution on cotton was placed at the bottom of the flask. A single 2-day old virgin male was released into the flasks on the first day of emergence. Adults emerged during the previous dark period were taken to be 0-day old adults. These adults were placed into the conical flasks and were used for experiment after two days. Two µl of different concentrations of synthetic sex pheromone hexane solution was applied to a small piece of filter paper (1.5 mm x 3 mm), attached to a stick with a sticky substance and behaviour was observed. The experiment was performed between 30 and 90 min. after light off. Behaviours observed were antennal movement, wing vibration and mating dance. Red-light of 10 W was used to observe the behaviour of DBM. Individual insects were used for the experiment only one time throughout the experiment. All observations were conducted in bioassay room (1.8 m x 1.8 m x 2 m) which is installed with a ventilation system. Data were analyzed by *t*-test using a personal computer NEC PC9801VM (Nippon Electric Co. Ltd., Tokyo, Japan).

Results and discussion

Data of the amounts of radioactivity recovered from the larvae, 96 hr. following feeding them on treated leaves, are given in *Table 1*. The detected amounts of metabolites in the resistant strain were higher than those detected in the susceptible one. The amounts of two metabolites which are products by carboxyamidase were two to three times higher in resistant strain than in susceptible one. These differences indicate that chlorfluazuron metabolism was higher in resistant strain than in the susceptible one.

Addition of TPP had almost no effect on the chlorfluazuron metabolism in the susceptible strain. On the other hand, in the resistant strain, the percentage of detected amounts of major metabolites i.e. 2,6-difluorobenzoic acid and 2,6-difluorobenzamide have decreased to almost the same levels, detected in the susceptible strain, after addition of TPP *(Table 1)*. These results are more or less in agreement with the results obtained in toxicological studies, where TPP showed high synergistic activity in resistant strains and not in susceptible ones (Fahmy, 1993).

When carboxyamidase activity was assessed *in vitro* using acetyl *p*-nitroanilide as a substrate, susceptible strains showed two to three times lower

Table 1. *In vitro* metabolism of ^{14}C-chlorfluazuron at 96 hr after treatment to the third instar DBM larvae of chlorfluazuron susceptible and resistant TL strains with and without TPP

		Percentage of ^{14}C-radioactivity recovered (± SD)			
		TL(S)		TL(R)	
Metabolites	Rf	-----	TPP	-----	TPP
Polar metabolies	0.00	5.0 ± 1.2	4.6 ± 0.5	7.6 ± 0.9	5.3 ± 0.8
2,6-Difluoro-benzoic acid	0.60	1.7 ± 0.1	2.1 ± 0.1	5.8 ± 0.8	2.4 ± 0.3
2,6-Dfluoro-benzamide	0.68	1.6 ± 0.1	1.6 ± 0.1	5.1 ± 0.5	1.9 ± 0.3
Chlorfluazuron	0.88	90.4 ± 1.2	90.7 ± 0.5	80.6 ± 0.7	89.2 ± 0.3
Others	---	1.2 ± 0.2	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.5
Total recovery % ± SD		82.9 ± 2.2	90.1 ± 3.1	89.2 ± 2.7	87.5 ± 1.9

Table 2. Carboxyamidase activities of chlorfluazuron susceptible and resistant strains of DBM

Strain	Carboxyamidase activity (10^{-4}umole/min/mg protein)
OSS (S)	1.108 ± 0.184
BKS (S)	1.327 ± 0.862
BKR (R)	2.854 ± 0.542
TLS (S)	1.346 ± 0.213
TLR (R)	3.08 ± 1.434
Hyogo (R)	2.954 ± 0.683
Kagoshima (R)	2.714 ± 1.105

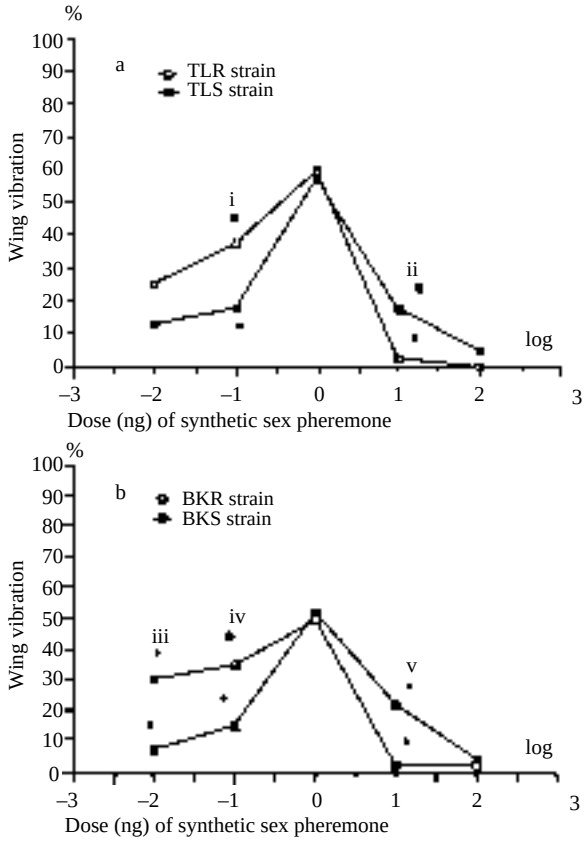


*Figure 1. Effects of synthetic sex pheromone on wing vibration of chlorfluazuron resistant and susceptible strains of DBM moth. *indicates there is a significant difference between chlorfluazuron resistant and susceptible strains of DBM (t-test, i-p=0.0005, ii-p=0.008, iii-p=0.009, iv-p=0.006, v-p=0.001).*

activities than resistant ones *(Table 2)*. Although degradation of other benzoylphenyl urea compounds such as diflubenzuron and teflubenzuron by resistant insect species is well documented (Lin *et al.*, 1989; El Saidy *et al.*, 1989; Van Laecke and Degheele, 1991), chlorfluazuron was reported to be highly stable to enzymatic degradation in many insects (Guyer and Newmann, 1988; Gazit *et al.*, 1989).

The difference in chlorfluazuron metabolism between the susceptible and the resistant strains observed in this study seems to be insufficient to explain the large difference in chlorfluazuron resistance levels (more than 300-fold). This might reflect the necessity of more investigation on the pattern of metabolite distribution in both larval body and the excreta separately, since the amount of unchanged chlorfluazuron incorporated inside the larval body, which is actually unknown, might differ between the susceptible and resistant insects. If the amount or speed of chlorfluazuron to reach the target is very small or slow, even a small difference in activity of carboxyamidase has an ability to cause a big difference in resistance level. If whole chlorfluazuron is degraded in resistant insects before it reaches to the target site, the small difference in enzyme activity can cause a big difference in resistance level.

When the synthetic sex pheromone was introduced into the flask, the rate of responses between chlorfluzruron resistant and susceptible strains of DBM showed a bell shape depending on the doses. There existed an optimal dose for insects to respond to the sex pheromone. Under the experimental condition, 1 ng of synthetic sex pheromone showed highest response. There were statistically significant differences in wing vibration (wing fanning) response between chlorfluazuron resistant and susceptible TL strains, at 0.1 ng (*t*-test, p=0.0005) and 10 ng (*t*-test, p=0.008). Between the chlorfluazuron resistant and susceptible BK strains, statistically significant differences were observed at 0.01 ng (*t*-test, p=0.009), 0.1 ng (*t*-test, p=0.006) and 10 ng (*t*-test, p=0.001) *(Figure 1)*. Chlorfluazuron susceptible DBM moths showed higher response in wider range of sex pheromone dose than the chlorfluazuorn resistant ones. At higher dose conditions, moths of resistant strains respond more strongly, however. at lower dose conditions, moths of susceptible strains responded more strongly. These results clearly confirmed the results reported by Mimori and Miyata (unpublished observation) who used insecticide resistant and susceptible strains with different genetic backgrounds.

The results obtained here suggested several interesting points. One is that chlorfluazuron susceptible DBM males can find females more efficiently than chlorfluazuron resistant males. This can partially explain the instability of chlorfluazuron resistance in the DBM. Secondly, when communication disruption method by synthetic sex pheromone was applied to control DBM (Talekar, 1992), chlorfluazuron susceptible males were more seriously affected than chlorfluazuron resistant ones. The similar observation was also found in pyrethroid resistant *Heliothis virescens* (Campanhola *et al.*, 1992). Regarding the sex pheromone sensitivity, further studies are needed to clarify whether there is any differences in sex pheromone synthesis, expiration, etc.

Acknowledgments

The authors wish to express sincere gratitude to Drs. T. Saito, T. Tanaka, T. Itioka and T.M. Brown for their valuable suggestion throughout this study. Thanks are also given to a joint DBM research group of Department of Entomology, Kasetsart University and Department of Agriculture, Thailand, Dr. S. Kono, Plant Protection Division, Hyogo Prefecture and Dr. A. Tanaka, Kagoshima Prefectural Agricultural Experiment Station for supplying the DBM strains

used. Thanks are also due to Nagoya Office for Plant Quarantine, the Ministry of Agriculture, Forestry and Fisheries, Japan for the permission to import DBM strains (Permission No. H1-1644). This work was supported in part by a Grand-in-Aid for Scientific Research Work No. 07556014 of the Ministry of Education, Science and Culture, Japan, Japan Society for the Promotion of Science, Chemical Materials Research and Development Foundation and Nagoya University Research Foundation.

References

Campanhola, C., McCutchen, B. F., Baehrecke E. H. and Plapp, Jr. F. W. (1991). Biological constraints associated with resistance to pyrethroids in the tobacco budworm (Lepidoptera: Noctuidae). *J. Econ. Entomol.* ***84:*** 1404–1411.

El-Saidy, M. F., Auda, M. and Degheele, D. (1989). Detoxification mechanisms of diflubenzuron and teflubenzuron in the larvae of *Spodoptera littoralis* (Boisd.). Pestic. *Biochem. Physiol.* ***35:*** 211–222.

Fahmy, A. R. (1993). Studies on the mechanism of resistance to chlorfluazuron in the diamondback moth (*Plutella xylostella* (L.)). PhD thesis for Graduate School of Agricultural Sciences, Nagoya University. 114pp.

Fahmy, A. R. and Miyata, T. (1992). Reversion of chlorfluazuron resistance after release from selection pressure in the diamondback moth, *Plutella xylostella*. *J. Pesticide Sci.* ***17:*** 83–85.

Fahmy, A. R., Sinchaisri, N. and Miyata. T. (1991). Development of chlorfluazuron resistance and pattern of cross resistance in the diamondback moth, *Plutella xylostella*. *J. Pesticide Sci.* ***16:*** 665–672.

Gazit, Y., Ishaaya, I. and Perry, A. S. (1989). Detoxification and synergism of diflubenzuron and chlorfluazuron in the red flour beetle *Tribolium castaneum*. Pestic. *Biochem. Physiol.* ***34:*** 103–110.

Guyer, W. and Newmann, R. (1988). Activity and fate of chlorfluazuron and diflubenzuron in the larvae of *Spodoptera littoralis* and *Heliothis virescens*. *Pestic. Biochem. Physiol.* ***30:*** 166–177.

Lin, J. G., Hung, C. F. and Sun, C. N. (1989). Teflubenzuron resistance and microsomal monooxygenases in larvae of the diamondback moth. *Pestic. Biochem. Physiol.* ***35:*** 20:25.

Noppun, V., Miyata, T. and Saito, T. (1983). Susceptibility of four strains of the diamondback moth, *Plutella xylostella* L. J. Pesticide Sci. 8:595–599.

Saito, T., Miyata, T., Sinchaisri, N. and Vattanatangum, A. (eds.) (1995). Management of Brown Planthopper and Resistance of diamondback moth. Nagoya University Cooperative Press, Nagoya, 161pp.

Sinchaisri, N., Chomkwah, S., Saito, T. and Miyata, T. (1989). Increasing of susceptibility of insect growth regulators of R-strain diamondback moth, *Plutella xylostella* L. 1st Asia-Pacific Conf. Entomol., Chaing Mai, Abstract p.231.

Talekar, N. S. (ed.) (1992). Diamondback Moth and Other Crucifer Pests. Asian Vegetable Research and Development Center, Taiwan, 603pp.

Talekar, N. S. and Griggs, T. D. (eds.) (1986). Diamondback Moth Management. Asian Vegetable Research and Development Center, Taiwan, 471pp.

Van Laecke, K. and Degheele, D. (1991). Detoxification of diflubenzuron and teflubenzuron in the larvae of the beet armyworm (*Spodoptera exigua*) (Lepidoptera: Noctuidae). *Pestic. Biochem. Physiol.* ***40:*** 181–190.

Woods, M. j., Findlater, J. D. and Orsi, B. A. (1979). Kinetic mechanism of the aliphatic amidase from *Pseudomonas aeruginosa*. *Biochem. biophys. Acta* ***567:*** 225–237.

Characterisation of knockdown resistance to pyrethroid insecticides in *Plutella xylostella*

T. H. Schuler[1], D. Martinez-Torres[1], A. J. Thompson[2], I. Denholm[1], A. L. Devonshire[1], I. R. Duce[2] and M. S. Williamson[1]

[1]*Department of Biological and Ecological Chemistry, IACR-Rothamsted, Harpenden, AL5 2JQ, United Kingdom*

[2]*Department of Life Science, University of Nottingham, Nottingham, NG7 2RD, United Kingdom*

Abstract

A combination of toxicological, electrophysiological and molecular studies confirmed target site insensitivity (often termed knockdown resistance or *kdr*) to be at least partly responsible for high and stable pyrethroid resistance in a Taiwanese strain of the diamondback moth, *Plutella xylostella*. Non-synergizable cross-resistance to a range of pyrethroids and DDT, as well as incompletely recessive autosomal inheritance of the resistance trait, provided indirect evidence for the presence of *kdr* in this strain. A larval neuromuscular preparation was used to assess spontaneous miniature excitatory post-synaptic potentials (mEPSP) and evoked EPSP's in response to varying concentrations of the type II pyrethroid deltamethrin. Intracellular recordings revealed a pyrethroid-induced increase in mEPSP activity and a decline in the EPSP amplitude, responses which were induced at considerably higher concentrations in resistant larvae when compared to larvae of a susceptible standard strain. These findings were supported by the detection of an amino acid substitution in the voltage-sensitive sodium channel (the primary target site of pyrethroids) of the resistant strain, which has previously been shown to correlate with *kdr* in the housefly, *Musca domestica*.

Key words: *Plutella xylostella*, pyrethroids, knockdown resistance (*kdr*), neurophysiology, sodium channel gene

Introduction

The ability of the diamondback moth, *Plutella xylostella* (L.), (Lepidoptera: Yponomeutidae) to develop high levels of resistance to pyrethroid insecticides is well documented (Talekar, 1992). Detoxification by mixed function oxidases (mfo) has been considered to be the major mechanism involved in this type of resistance (Sun, 1992) although indirect evidence has also accumulated for the widespread occurrence of reduced nerve sensitivity to pyrethroids (Cheng, 1988; Liu *et al.*, 1981, 1982a, 1982b; Miyata *et al.*, 1992). This type of resistance was originally described for houseflies (*Musca domestica* L.) under the name knockdown resistance (*kdr*) (Busvine, 1951; Milani, 1954). *Kdr* and a second more potent type of nerve insensitivity, termed *super-kdr,* are thought to result from structural changes in the voltage-gated sodium channel, the primary target site for pyrethroids and DDT in the insect nervous system (Bloomquist, 1996). Recently, two mutations in the *para*-type sodium channel gene of the housefly have been linked to the occurrence of *kdr* and *super-kdr* resistance in this insect (Williamson *et al.*, 1996). Moreover, one of these mutations has also been reported in a *kdr* strain of the German cockroach (*Blattella germanica* L.) (Miyazaki *et al.*, 1996). Although nerve insensitivity to pyrethroids has been investigated in other insects (reviewed in Soderlund and Bloomquist, 1990), housefly and German cockroach remain the only species where this type of resistance has been characterised at the molecular level.

The first direct evidence for the presence of *kdr*-type resistance in the diamondback moth was provided by Hama *et al.* (1987) who demonstrated electrophysiologically a reduced sensitivity of the central nerve cord in larvae of pyrethroid resistant Japanese strains. We now report evidence for the presence of *kdr*-type resistance in a pyrethroid resistant strain of the diamondback moth from Taiwan based on results obtained with toxicological, electrophysiological and molecular techniques.

Toxicological studies

Pyrethroids and DDT in acetone were applied topically to fourth instar larvae of two diamondback moth strains. The susceptible strain (Rothamsted) had been maintained in laboratory culture for over 30 years without insecticide selection. The pyrethroid resistant strain, FEN (formerly FP), was obtained from C. N. Sun in 1995. FEN was collected in Taiwan in 1983 and subsequently selected with fenvalerate (Chen and Sun, 1986). Topical bioassays at Rothamsted with a range of pyrethroids confirmed a high level of pyrethroid resistance previously reported in this strain (Chen and Sun, 1986; Yao *et al.*, 1988). Resistance to pyrethroids possessing an α-cyano-3-phenoxybenzyl alcohol moiety, such as fenvalerate and deltamethrin, was impossible to quantify with resistance factors exceeding 10,000 *(Table 1)*. Although mortality was

Table 1. Susceptibility to pyrethroids and DDT of larvae of a susceptible (Rothamsted) and a resistant (FEN) diamondback moth strain

Compound	Strain		RF[b]
	Rothamsted LD_{50} (μg/larva)[a]	FEN LD_{50} (μg/larva)	
Fenvalerate	0.003	100μg=0%	>33 000
Deltamethrin	0.001	10μg=6%	>10 000
Bioresmethrin	0.006	c. 10	1 700
Cismethrin	0.004	c. 10	5 000
DDT	0.92	10μg=0%	>10

[a]Topical application to fourth instar larvae, assessment of mortality five days after treatment.
[b]Resistance factor = LD_{50} of FEN/LD_{50} of Rothamsted strain

still observed with pyrethroids based on a 5-benzyl-3-furylmethyl alcohol, such as bioresmethrin and cismethrin, the resistance factors were still extremely high (1700–5000) *(Table 1)*.

Piperonyl butoxide (PB) and a range of other mfo inhibitors, including PBX, Niagara 16824, TCPB and *m*-nitro propargyl ether, were tested with fenvalerate. When applied topically to larvae 30–60 min prior to the insecicides, synergism was unexpectedly low. PB was the most effective synergist but only resulted in up to 59% mortality at a dose of 100 μg fenvalerate per larvae (compared to a LD_{50} of 0.003 μg for susceptible larvae). In contrast, Chen and Sun (1986) had been able to reduce the LD_{50} of FEN for fenvalerate (spray application) from >100 mg/ml to 5.5 mg/ml (synergism ratio of >18) by pretreatment with PB. At present it remains unclear why mfo inhibitors were ineffective in synergising pyrethroids in our study. DDT was also ineffective against FEN *(Table 1)*. Resistance to DDT was not synergisable by PB or FDMC, an inhibitor of DDT-dehydrochlorinase.

Virgin adults of the two strains were crossed and larvae of the F_1 generation tested by topical application of insecticides. LD_{50} values were drastically lower than for the FEN strain, with resistance factors of 20 and <5 for fenvalerate and bioresmethrin, respectively. There was no significant difference between reciprocal crosses, leading to the conclusion that pyrethroid resistance in FEN was a largely recessive autosomal trait, confirming previous results with other diamondback moth strains (Hama *et al.*, 1987; Liu *et al.*, 1981; Kim *et al.*, 1991, Miyata *et al.*, 1992; Motoyama *et al.*, 1992). No selection pressure was applied to the FEN strain after its arrival in the UK in 1995, and no decrease in resistance has since been observed over 25 generations, indicating a high level of homozygosity of the resistance genes.

Electrophysiological assay for nerve insensitivity

The central nerve cord and the ventral internal lateral (VIL) muscles of decapitated fourth instar diamondback moth larvae were exposed by dissection under saline. Stimulation of the segmental ganglion using a suction electrode evoked excitatory post-synaptic potentials (EPSP) which were recorded intracellularly in the adjacent segmental muscle.

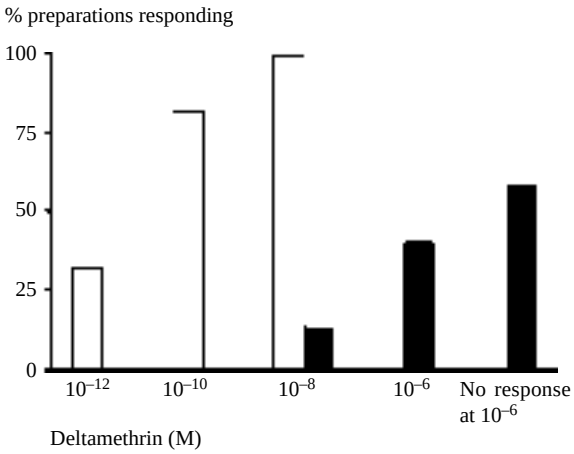


Figure 1. Distribution of neuronal responses of fourth instar diamondback moth larva nerve muscle preparations treated with increasing doses of deltamethrin (❑ Rothamsted strain, ■ FEN strain, n=22)

Preparations were perfused with concentrations of deltamethrin ranging from 10^{-12}M to 10^{-6}M for 10 min at each concentration. Recordings revealed that deltamethrin induced a decline in the EPSP amplitude and an increase in miniature EPSP activity. Over 80% of susceptible larvae responded at a concentration of 10^{-10}M deltamethrin or lower *(Figure 1)*. Much higher concentrations were necessary to elicit a response in FEN larvae. Only 14% of FEN larvae reacted at 10^{-8}M deltamethrin rising to only 41% at the highest dose (10^{-6}M). Sixty percent of FEN larvae did not respond to the highest deltamethrin dose. EC_{50} values were estimated at 10^{-11}M and 10^{-6}M for the susceptible and the FEN strains, respectively, a resistance ratio of over 300,000-fold. The electrophysiological assay thus demonstrated a high level of nerve insensitivity in the FEN strain.

Molecular study

Molecular cloning studies of the *para*-type sodium channel gene in the housefly (Williamson *et al.*, 1996) and German cockroach (Miyazaki *et al.*, 1996) have identified two amino acid changes in the channel sequence that correlate with *kdr* resistance phenotypes. Both changes are located in the domain II region of the channel and involve: 1) a leucine to phenylalanine (Leu to Phe) substitution in the hydrophobic IIS6

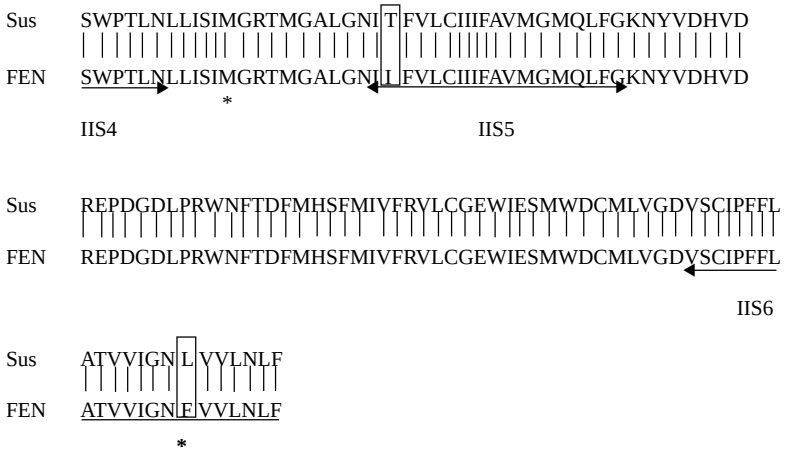


Figure 2. Alignment of amino acid sequences of the IIS4–IIS6 sodium channel region of the Rothamsted (Sus) and the pyrethroid resistant FEN strain of the diamondback moth. Mutations in the diamondback moth are highlighted in boxes whereas mutations associated with kdr resistance in the housefly are indicated by stars.

transmembrane segment (found in *kdr* and *super-kdr* houseflies and *kdr* cockroaches), and 2) a methionine to threonine (Met to Thr) substitution within the intracellular loop between IIS4 and IIS5 (only found in *super-kdr* houseflies). We therefore examined this region of the sodium channel in the Rothamsted and FEN strains of diamondback moth to determine whether either or both of these changes were also associated with resistance in this species.

Degenerate PCR primers were based on conserved sequences of vertebrate and invertebrate sodium channel, enabling selective amplification of the IIS4–IIS6 region of the channel from any insect species. Total RNA was extracted from 4th instar larvae of Rothamsted and FEN strains and cDNA synthesised as the template for PCR. cDNA was used in preference to genomic DNA because the *para* gene contains several introns in this region (Loughney *et al.*, 1989), one of which disrupts the 3' primer site. Following two rounds of PCR with the degenerate primers, discrete fragments of the expected size (350bp) were obtained for both strains which were later cloned and sequenced. The fragments encoded amino acid sequences with close identity to the corresponding region of housefly (94%) and cockroach (89%) *para* sodium channels. An alignment of the Rothamsted and FEN sequences *(Figure 2)* revealed two amino acid differences in this region. One was the same Leu (Rothamsted) to Phe (FEN) substitution in IIS6 channel sequence as has been previously reported for housefly and cockroach *kdr* strains *(Figure 2)*. The second change, however, was different to that in *super-kdr* housefly strains, revealing a threonine (Thr) (Rothamsted) to isoleucine (Ile) (FEN) substitution at the beginnning of the IIS5 segment *(Figure 2).*

The identification of the same Leu to Phe substitution in the sodium channel sequence of FEN insects further consolidates the association of this mutation with *kdr*-type resistance and indicates an important role in conferring the nerve insensitive phenotype of this strain. The significance of the second mutation (Thr to Ile) is still unclear since this has not been reported previously and may simply represent a polymorphism with no relevance to the pyrethroid resistance. However, its close proximity to the housefly Met to Thr substitution, and the high degree of conservation of the Thr residue in other sodium channel sequences (vertebrate and invertebrate), may indicate a more direct role for this mutation in contributing to the strong resistance of the FEN strain.

Conclusion

The high resistance of the FEN strain to pyrethroids is at least partially based on a marked decrease in nerve sensitivity to these compounds. Knockdown resistance in this strain is characterised by cross resistance to pyrethroids and DDT, little or no synergism of pyrethroid and DDT resistance, incompletely recessive autosomal inheritance, over 300 000-fold reduced nerve sensitivity in an electrophysiological assay, and the presence of the housefly *kdr*-type mutation in the otherwise highly conserved sodium channel gene. The low level of synergism of pyrethroids by mfo inhibitors, although in line with the presence of knockdown resistance, does not conform with previous work on the FEN strain and needs further investigation. This work presents the first report of the housefly-*kdr* mutation (Leu to Phe) in a crop pest species. We are presently investigating the distribution of this mutation in the diamondback moth by analysing strains from different parts of the world. This will also help to determine whether the second mutation (Thr to Ile) correlates with the *kdr* resistance phenotype in this insect.

Acknowledgements

We would like to thank Prof C. N. Sun for providing the FEN strain.

References

Bloomquist, J. R. (1996). Ion channels as targets for insecticides. *Annual Review of Entomology* ***41:*** 163-190

Busvine, J. R. (1951). Mechanism of resistance to insecticide in houseflies. *Nature* ***168:*** 193-195

Chen, J. S. and Sun, C. N. (1986). Resistance of diamondback moth (Lepidoptera: Plutellidae) to a combination of fenvalerate and piperonyl butoxide. *Journal of Economic Entomology* ***79:*** 22-30

Cheng, E. Y. (1988). Problems of control of insecticide-resistant *Plutella xylostella*. *Pesticide Science* ***23:*** 177-188

Hama, H., Kono, Y. and Sato, Y. (1987). Decreased sensitivity of central nerve to fenvalerate in the pyrethroid-resistant diamondback moth, *Plutella xylostella* Linne (Lepidoptera: Yponomeutidae). *Applied Entomology and Zoology* ***22:*** 176-180.

Kim, G. H., Lee, J. H. and Cho, K. Y. (1991). Inheritance of fenvalerate resistance in the diamondback moth, *Plutella xylostella* Linne (Lepidoptera: Yponomeutidae). *Korean Journal of Applied Entomology* ***30:*** 106-110.

Liu, M. Y., Sun, C. N. and Huang, S. W. (1982a). Absence of synergism of DDT by piperonyl butoxide and DMC in larvae of the diamondback moth (Lepidoptera: Yponomeutidae). *Journal of Economic Entomology* ***75:*** 964-965

Liu, M. Y., Tzeng, Y. J. and Sun, C. N. (1981). Diamondback moth resistance to several synthetic pyrethroids. *Journal of Economic Entomology* ***74:*** 393-396

Liu, M. Y., Tzeng, Y. J. and Sun, C. N. (1982b). Insecticide resistance in the diamondback moth. *Journal of Economic Entomology* ***75:*** 153-155

Loughney, K., Kreber, R. and Ganetzky, B. (1989). Molecular analysis of the para locus, a sodium channel gene in *Drosophila*. *Cell* ***58:*** 1143-1154

Milani, R. (1954). Comportamento mendeliano della resistenza alla azione abbattante del DDT: correlazione tran abbattimento e mortalia in *Musca domestica. L. Riv Parasitol* ***15:*** 513-542

Miyata, T., Noppun, V. and Saito, T. (1992). Inheritance of resistance to phenthoate and fenvalerate in diamondback moth and management of insecticide resistance. *Proceedings of the Second International Workshop on the Diamondback Moth and Other Crucifer Pests*, Tainan, 1990, pp. 477-484

Miyazaki, M., Ohyama, K., Dunlap, D. Y. and Matsumura, F. (1996). Cloning and sequencing of the *para*-type sodium channel gene from susceptible and *kdr*-resistant German cockroaches (*Blattella germanica*) and house fly (*Musca domestica*). *Molecular and General Genetics.* ***252:*** 61-68

Motoyama, N., Suganuma, T. and Maekoshi, Y. (1992). Biochemical and physiological characteristics of insecticide resistance in diamondback moth. *Proceedings of the Second International Workshop on the Diamondback Moth and Other Crucifer Pests*, Tainan, 1990, pp. 411-418

Soderlund D. M. and Bloomquist J. R. (1990). Molecular mechanisms of insecticide resistance. In *Pesticide Resistance in Arthropods* (eds R. T. Roush and B. E. Tabashnik), pp. 58–96, Chapman and Hall, New York

Sun, C. N. (1992). Insecticide resistance in diamondback moth. *Proceedings of the Second International Workshop on the Diamondback Moth and Other Crucifer Pests*, Tainan, 1990, pp. 419-426

Talekar, N. E. (1992). *Proceedings of the Second International Workshop on the Diamondback Moth and Other Crucifer Pests*, Tainan 1990, 603 pp.

Williamson, M. S., Martinez-Torres, D., Hick, C. A. and Devonshire, A. L. (1996). Identification of mutations in the housefly *para*-type sodium channel gene associated with knockdown resistance (*kdr*) to pyrethroid insecticides. *Molecular and General Genetics* ***252:*** 51-60

Yao, M. C., Hung, C. F. and Sun, C. N. (1988). Fenvalerate resistance and aldrin epoxidation in larvae of the diamondback moth. *Pesticide Biochemistry and Physiology* ***30:*** 272–278

Glutathione *S*-transferases and insecticide resistance of diamondback moth

Y.E. Yao, H.H. Huang, C.Y. Wu, S.W. Chiang, N.T. Hu[1] and C.N. Sun

Department of Entomology and [1]Agricultural Biotechnology Laboratories
National Chung-Hsing University, Taichung, Taiwan 40227 Republic of China

Abstract

The role of glutathione *S*-transferase (GST, EC 2.5.1.18) in the degradation of and resistance to some insecticides of diamondback moth (DBM) larvae has been confirmed through the purification and characterization of its four isozymes from this notorious insect pest of cruciferous vegetables. Two immuno-related isozymes, GST-3 and GST-4, exhibit apparent substrate preference for 1,2-dichloro-4-nitrobenzene, a model substrate, and several organophosphorus (OP) insecticides, i.e., parathion, methyl parathion and paraoxon. GST-3 exists in higher proportion in DBM strains showing OP resistance while GST-4 is observed only in DBM selected with teflubenzuron, a chitin synthesis inhibitor. With degenerate primers based on the N-terminal amino acid sequences of GST-3 and a CNBr-cleaved fragment of this protein, we have obtained a 128 bp DNA product from reverse transcription polymerase chain reaction (PCR). Screening a cDNA library prepared from a methyl parathion-resistant DBM strain with this PCR product as probe has yielded two clones. These two clones are proved to be the same with the longer one of 809 pb encoding a protein, pxGST3, of 216 amino acids with a predicted molecular mass of 24 kDa and a pI of 9.37. An alignment with the nine insect GSTs already cloned shows that PxGST3 shares the highest (46.3%) amino acid sequence identity to one of the two GSTs (MsGST1) of *Manduca sexta*. PxGST3 is believed to be the first cloned insect GST with a well-defined role in insecticide resistance.

Key words: Diamondback moth, glutathione S-transferase, insecticide resistance

Introduction

Glutathione *S*-transferases (GSTs, EC2.5.1.18) are a family of enzymes that catalyze the nucleophilic attack of the sulfur atom of glutathione on the electrophilie center of many chemical compounds (Mannervik and Danielson, 1988). The GSTs, in addition to their enzymatic activities, can bind with high affinity a variety of hydrophobic compounds (Daniel, 1993). These enzymes are abundant and widely distributed in most forms of life. In insects, they have been known to detoxify some organophosphorus (OP) and organochlorine insecticides (Dauterman, 1985), and play an important role in insect resistance to these compounds (Oppenoorth, 1985). The GSTs are also involved in insect detoxication of plant allelochemicals and thus related to host plant range in phytophagous insects (Yu, 1992).

The papers we presented during the First and the Second Workshops (Sun *et al.*, 1986; Sun, 1992) dealt with the insecticide resistance problems – what we knew then and what we could do in terms of resistance management. Talekar and Shelton (1993) pointed out that for establishing sustainable management systems, multiple-component strategies must be adopted and new technologies must complement traditional ones. Although in the future, chemical insecticides (including probably the microbial agent Bt) may not be the dominating element in DBM control as in the past three decades, they will remain an indispensable part of the management system. As studies of insecticide resistance have entered the molecular age (Mullin and Scott, 1992) and tremendous progress has been made, researchers will have to take on a new look at this phenomenon in DBM. In this paper we will review our work on glutathione *S*-transerases of DBM, and then describe briefly results on the molecular cloning of a GST gene involved in insecticide resistance of this insect.

Glutathione S-Transferases of DBM

Kao and Sun (1991) first demonstrated that glutathione conjugation was a major detoxifying reaction for parathion and methyl parathion in DBM, and a considerably higher degradation of both insecticides was found in the resistant than in the susceptible strains. They proposed the existence of isozymes of GST in DBM. Subsequently four GST isozymes have been isolated and purified from DBM larvae with affinity chromatography followed by cation exchange chromatography (Chiang and Sun, 1993; Ku *et al.* 1994). The biochemical and toxicological characteristics of these GST isozymes are given in *Table 1* and *Figure 1*. They are homodimers with subunits of molecular mass ranging from ca. 23.6 to 27.1 kDa. All except GST-1 are basic proteins with pIs >8.0 *(Table 1)*. In contrast to purified housefly GSTs which prefer conjugation with methyl group (Motoyama, 1982), all four GSTs of DBM appear to conjugate only with the aryl group of both parathion and methyl parathion (Chiang and Sun, 1993), and they are unable to catalyze the detoxication of some other OPs known as GST substrates, such as fenitrothin,

Table 1. Subunit molecular mass and pI of purified GST isozymes from DBM larvae

Isozyme	pI	Subunit
GST-1	ca. 4.8	27.1
GST-2	8.2	23.6
GST-3	ca. 8.7	26.5
GST-4	ca. 8.9	26.6

Adapted from Ku *et al.,* (1994)

diazinon, teterachlor-vinphos and azinphosmethyl (Kao and Sun, 1991).

An interesting finding regarding these GSTs is their distinct substrate preferences *(Figure 1)*. Toward the two model substrates studies, i.e., 1-chloro-2,4-dinitrobenzene (CDNB) and 1,2-dichloro-4-nitrobenzene (DCNB), GST-2 has considerably higher activity toward CDNB then the others while GST-3/GST-4 exhibit 8 to 20-fold higher activity toward DCNB. The latter two GST isozymes can degrade parathion, methyl parathion and paraoxon 13 to 70-fold more effectively than GST-1/GST-2.

Figure 2 shows the GST profiles of several strains of DBM (Ku *et al.,* 1994). While no GST-3 is seen in the susceptible MT strain or fenvalerate-resistant FEN strain, this isoform is present in a methyl parathion-resistant MPA strain, a field-collected TC strain, a mixed-field MD strain as well as a teflubenzuron (TFB)-resistant CME strain. The absence of GST-3 is in accordance with the lack of cross-resistance to these OPs of FEN strain (Sun *et al.,* 1992). The somewhat lower proportion of GST-3 in MD strain could be related to the fact that this strain has been reared in the laboratory without any insecticide exposure. Among the six strains examined, CME strain is unique in terms of GST isozyme profile. Unexpectedly high GST activity toward both DCNB and methyl parathion has been noticed (Sun *et al.,* 1992). Its GST-3 content is low yet it contains an additional form, GST-4. This isozyme displays even stronger preference for the three OPs than GST-3 *(Figure 1)*, and yet why CME strain does not exhibit a level of methyl parathion resistance comparable to that of MPA strain (>450-fold) is still unclear. Preliminary results suggest that GST-4 might bind TFB specially to render it ineffective as a chitin synthesis inhibitor (Jung and Sun, unpublished data).

Molecular cloning of a GST gene involved in insecticide resistance of DBM

While GST-3 and GST-4 are highly immuno-related, polyclonal antiserum raised against GST-3 cross-reacts with GST-1 and GST-2 at least 40-fold less intensely than with the antigen (Ku *et al.,* 1994). GST-3 and GST-4 share at least identical first 20 amino acids at

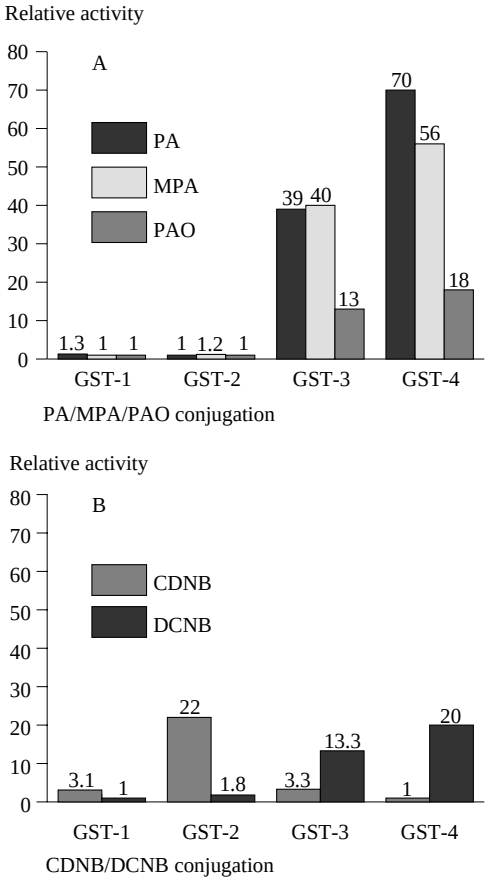


Figure 1. Substrate preference of purified GST isozymes of DBM. A. CNDB: 1-chloro-2,4-dinitrobenzene, and DCNB: 1,2-dichloro-4-nitrobenzene. B. PA: parathion, MPA: methyl parathion and PAO: paraoxon (Adapted from Ku et al. 1994)

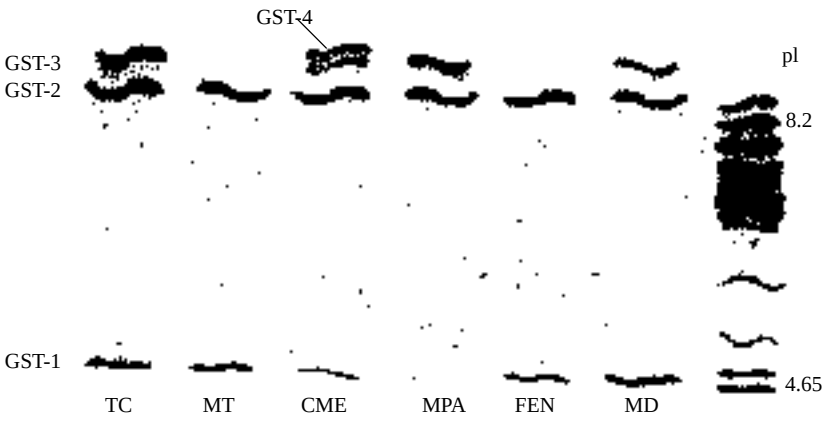


Figure 2. GST isoform profiles of several strains of DBM. TC: field strain, MT: susceptible strain, CME: teflubenzuron-resistant strain, MPA: methyl parathion-resistant strain, FEN: fenvalerate-resistant strain and MD: mixed field strain (Adapted from Ku et al., 1994)

Table 2. Percentage identities of DBM GST-3 with nine other insect GSTs

Group	Insect	PxGST3 % Identity
I	DmGST2	12.0
	AgGST2	12.2
	MsGST2	17.6
II	DsimGST	38.2
	DmGST1	37.1
	MdGST1	37.8
	MdGST2a	36.4
	DmGST27	33.0
	MsGST1	46.3

Dm: *Drosophila melanogaster*
AG: *Anopheles gambiae*
Ms: *Manduca sexta*
Md: *Musca domestica*
Px: *Plutella xylostella*

the N-terminus while GST-1 and GST-2 have distinctively different N-terminal amino acid sequences. Thus we speculate that GST-1, GST-2 and GST-3/GST-4 are products of independent genes.

We started our molecular studies on insecticide resistance of DBM by cloning the cDNA of GST-3, one of the two efficient OP-degrading isozymes. Degenerate primers synthesized according to the N-terminal amino acid sequences of GST-3 and a 16.1 kDa CNBr-cleaved fragment of GST-3 have been used in reverse transcription polymerase chain reaction (PCR). A 128 bp PCR product was obtained with mRNA of the MPA strain as template. It was subsequently used as the probe to screen a cDNA library prepared from MPA strain. Two identical clones of 765 and 809 bp, respectively, were obtained. The longer clone, pGTPx3, had an open reading frame of 648 bp encoding 216 amino acids (GenBank accession U66342). The calculated molecular mass and pI of this protein, PxGST3, are 24 kDa and 9.37.

The GST-3 of DBM is aligned with nine known GSTs from insects including *Drosophila melanogaster, D. simulans, Anopheles gambiae, Musca domestica and Manduca sexta (Table 2)* (Snyder *et al.*, 1995). Insect GSTs can be classified into two groups based on the percentage identities of their amino acids. PxGST3 shows the greatest identity, 46.3%, in amino acid sequence to the MsGST1 of *M. sexta*, the first and only other lepidopterous insect whole GST cDNAs have been cloned, and somewhat lower identity to the remaining five GSTs of the same group.

Previous studies on GSTs of housefly did not give conclusive results with regard to the roles of the cloned genes in insecticide resistance, though resistant strains were used (Fournier *et al.*, 1992; Wang *et al.*, 1991). PxGST3 is believed to be the first cloned insect GST with a well-defined role in OP resistance.

References

Chiang, F.M. and Sun, C.N. (1993). Glutathione transferase isozymes of diamondback moth larvae and their role in the degradation of some organophosphorus insecticides. *Pesticide Biochemistry Physiology* ***45:*** 7–14.

Daniel, V. (1993). Glutathione *S*-transferases: gene structure and regulation of expression. CRC Critical *Review of Biochemistry and Molecular Biology* ***28:*** 173–207.

Dauterman, W.C. (1985). Insect metabolism: extramicrosomal. *In:* Comprehensive Insect Physiology, Biochemistry and Pharmacology (eds. G.A. Kerket and L. I. Gilbert) Vol. 12, pp. 713–730. New York: Pergamon Press.

Fournier, D., Bride, J.M., Poirie, M., Berge, J.B. and Plapp, Jr., F.W. (1992). Insect glutathione S-transferases: biochemical characteristics of the major forms from housefly susceptible and resistant to insecticides. *Journal of Biological Chemistry* ***167:*** 1840–1845.

Kao, C.N. and Sun, C.N. (1991). *In vitro* degradation of some organophosphorous insecticides by susceptible and resistant diamondback moth. *Pesticide Biochemistry and Physiology* ***41:*** 132–141.

Ku, C.C., Chiang, F.M., Hsin, C.Y., Yao, Y.E. and Sun, C.N. (1994). Glutathione transferase isozymes involved in insecticide resistance of diamondback moth larvae. *Pesticide Biochemistry and Physiology* ***50:*** 191–197.

Mannervik, B. and Danielson, U.H. (1988). Glutathione transferase: structure and catalytic activity. *CRC Critical Review of Biochemistry* ***23:*** 281–334.

Motoyama, N. (1982). Characterization of glutathione S-transferases in relation to azinphosmethyl resistance. *J. Pesticide Science* ***7:*** 415–421.

Mullin, C.A. and Scott, J.G. (1992). Biomolecular basis for insecticide resistance. Classification and comparison. *In Molecular Mechanism of Insecticide Resistance. Diversity among Insects* (eds. C.A. Mullin and J.G. Scott). pp. 1–13. American Chemical Society (ACS).

Oppernoorth, F.J. (1985). Biochemistry and genetics of insecticide resistance. *In:* Comprehensive Insect Physiology, Biochemistry and Pharmacology (eds. G.A. Kerket and L.I. Gilbert) Vol. 12, pp. 731–774. New York: Pergamon Press.

Synder, M.J., Walding, J.K. and Feyereisen, R. (1995). Glutathione *S*-transferase from larval *Manduca sexta* midgut: sequence of two cDNAs and enzyme induction. *Insect Biochemistry and Moleculr Biology* ***25:*** 455–465.

Sun, C.N., Wu, T.K., Chen, J.S. and Lee, W.T. (1986). Insecticide resistance in diamondback moth. *In Proceedings of the First International Workshop on Diamondback Moth Management* (ed. N.S. Talekar). Asian Vegetable Research and Development Center (AVRDC), Tainan, 1985, pp. 359–371.

Sun, C.N. (1992). Insecticide resistance in diamondback moth. In *Diamondback Moth and Other Crucifer Pests: Proceedings of the Second International Workshop* (ed. N.S. Talekar). AVRDC, Tainan, 1990, pp. 419–426.

Sun, C.N. Tsai, Y.C. and Chiang, F.M. (1992). Resistance in the diamondback moth to pyrethroids and benzoylphenyl ureas. *In Molecular Mechanisms of Insecticide Resistance. Diversity among Insects* (eds. C.A. Mullin and J.G. Scott). pp. 149–167. ACS.

Talekar, N.S. and A. Shelton. ((1993). Biology, ecology and management of the diamondback moth. *Annual Review of Entomology* ***38:*** 275–301.

Wang, J.Y., Maccommas, S. and Syvanen, M. (1991). Molecular cloning a glutathione *S*-transferase overproduced in an insecticide-resistant strain of the housefly *(Musca domestica). Molecular and General Genetics* ***227:*** 260–266.

Yu, S.J. (1992). Plant-allelochemical-adapted gluathione transferases in Lepidoptera. In *Molecular Mechanisms of Insecticide Resistance. Diversity among Insects* (eds. C.A. Mullin and J.G. Scott). pp. 174–190. ACS.

The historical failure of insecticide resistance management of the diamondback moth and the way forward

S. Uk[1] and J.G. Harris[2]

[1]Ciba-Geigy Ltd, Basle, Switzerland, (Zeneca Agrochemicals, Jealott's Hill Research Station, Berks., England)

[2]IRAC Field Crops & Vegetables Working Group, GIFAP asbl, Avenue Albert Lancaster 79a, 1180 Bruxelles, Belgium

Abstract

Resistance of diamondback moth (DBM) to insecticides in Southeast Asia remains notorious despite repeated efforts by the chemical industry and academia to manage it. New insecticides continue to lose their efficacy a few seasons after their introduction for use. Scientific knowledge on the DBM resistance profile and resistance mechanisms in the literature is voluminous. However, there is still a large gap between knowledge and practical implementation to avoid or delay resistance at the farm level. Farmers lack practical technical guidance on methods of pest control in crucifers. They often follow their innovative instinct by searching for new products from retailers for quick and effective solutions. Farmers may use an effective product continuously until it can no longer control the pests. In addition, the practice by farmers to ensure efficacy by mixing a cocktail of different products, often without a technical foundation for the mix, probably contributes further to aggravating the resistance problem. The risk of resistance to the mixture as well as to the new products may be accelerated.

At first sight, it is an almost insurmountable task to manage the resistance of DBM in Southeast Asia. The way forward in insecticide resistance management is a bottom-up approach, hand-in-hand with the farmers and retailers who are the starting point from where all concepts and techniques of Integrated Pest Management (IPM) and Insecticide Resistance Management (IRM) will be implemented. A commitment from the chemical industry, academia, governments, international organisations (financial donors, researchers, trainers, advisers) to work together, is essential in devising a common practical IRM strategy that will be favourably perceived and accepted by farmers and pesticide retailers. The chemical industry is ready for partnership with all concerned parties to arrive at a workable solution for the benefit of all.

Key words: Diamondback moth, insecticide resistance, IRM/IPM holism, implementation, partnership

Introduction

The notoriety of diamondback moth resistance to insecticides in the Far East and Southeast Asia needs little elaboration. All crucifer growers and vegetable entomologists are well aware of the destructive potential of this tiny lepidopterous pest when control measures fail. Talekar (1993) estimated an annual cost of US $1 billion world-wide to manage DBM on crucifers. DBM resistance was observed in Southeast Asia in the 1950's and gradually increased in seriousness as more and more insecticides lost their efficacy (e.g. Syed and Loke, 1995). In the last 15 years no new products have remained effective longer than 2–3 years after market introduction (Murai *et al.*, 1992; Adachi and Futai, 1992, etc.). Even complex molecules such as avermectin or Bt endotoxins (macromolecules) could not escape the resistance ability of DBM (Abro *et al.*, 1988; Shelton *et al.*, 1993; Martinez-Ramirez *et al.*, 1995; Tabashnik, 1994; Tang *et al.*, 1995a and 1995b).

Research knowledge on the extent of DBM resistance, the mechanisms of resistance and the scientific potential of resistance is sufficient to implement a set of practical recommendations at the farmer level. However, despite numerous scientific publications, local and international workshops and conferences, the practical reality in the field leaves much room for improvement. Implementation of IRM cannot be mere writings of procedures; one must go to the field and convince growers and pesticide retailers to adopt the recommendations. Thus, the recommended IRM tactics must be simple, convenient, relevant, and in the interest of retailers and farmers.

This paper hopes to reinforce the need to go beyond mere discussion and publications of recommendations.

Root of the DBM resistance problem

Farmer's attitude

Farmers, like anyone else, have a flair for trying innovative ways to solve problems. Innovation takes different forms under different circumstances – in the case of vegetable growers in the Far East and/or Southeast Asia, innovation often means searching for newly introduced products for pest control and using them until the arrival of other new compounds. This practice is inadvertently encouraged by pesticide retailers who are also proud to promote their new merchandises. Pesticide retailers and farmers tend to think that there will always be new products on the

market to replace those that have lost their efficacy. Superficially, they have been right so far. The last ten years saw the regular introduction of new insecticides for use on crucifers just as the existing products began to lose their effectiveness (real or perceived) on DBM. Examples of recently introduced compounds are diafenthiuron, avermectin, new Bt's (Centari®, Agree/ Turex, etc.), tebufenozide, fipronil and chlorfenapyr. The truth is that these new products are results of at least 10 years or more of expensive research and development work. New products with different modes of action are valuable tools that would help the implementation of IRM recommendations. On the other hand, these new products may lead retailers and farmers to a false sense of security. They may disregard recommendations (or plea) from academic, governmental or industrial researchers to use insecticides judiciously. Persuading farmers to believe and accept insecticide resistance management (IRM) is a challenge that governments, academia and industry must take up and pursue with patience and with a united front.

Technical constraints

Two main aspects inherent to the crucifer crops in tropical Asia make the implementation of IRM for DBM a very difficult task. As Prof. Sun pointed out at the Second International DBM Workshop (Sun, 1992):

1. Crucifers are grown year-round, thus providing an uninterrupted food supply for DBM to develop many overlapping generations per year (> 20 generations).
2. Different crucifer cultivars are attractive to many species of phytophagous insects; several of them may infest the crop from the beginning of the growing period. Depending on the pest species, the crop may require the use of an insecticide that contradicts insecticide resistance management for DBM. The implication of this is that separate, fragmented recommendation for each pest is doomed to failure; the entire pest complex must be studied and understood before devising a pest management model for the crop.

Market perception

The practice of grading harvested crucifers into different marketable categories does not help the implementation of IPM and IRM. The price differences that farmers receive from vegetable-wholesale dealers for their crops may encourage the farmer to use only a single most effective insecticide than is necessary in the hope of obtaining all Grade-A crops. Informal interviews have often brought to light that farmers spray their cabbage or Chinese kale every 3 to 5 days. The direct money or credit received from the buyers remains the strongest incentive for the small vegetable growers in Southeast Asia. One obstacle to IPM implementation caused by "cosmetic quality standard of produce" has been outlined in a review by Wearing (1988). Wearing assumed the cosmetic factor to be of low importance, even in fruit crops where unblemished fruit appearance is essential. The situation is reversed in the crucifers, whereby cabbages or Chinese kale with perforated leaves will fetch only a fraction of the price of their larger, attractively perfect counterparts. The attitude of merchants and consumers has to change from favouring good physical appearance in order for IPM/IRM to be practised universally with greater success.

Classical IRM process

IRM has no general recipe and each resistance case needs its own local solution (Uk *et al.*, 1995). IRM tactics must be based on local knowledge of the pest susceptibility status and crop management habit of farmers. However, all cases of IRM development will have in common the following steps:

- General survey and monitoring of pest susceptibility and resistance. This needs the development of suitable monitoring technique. Initial baseline data on susceptibility and resistance are the basis to further investigations.
- Determination of major resistance mechanisms.
- Investigation of cross resistance pattern amongst insecticides in use.
- Development of relevant IRM tactics that are simple and practical to local growers.
- Periodic updating of tactics as experience and new tools are available.

Research data from the above steps, although never all conclusive, should be sufficient to formulate and recommend IRM techniques for DBM (e.g. Zhu *et al.*, 1991; Cheng *et al.*, 1992; Saito *et al.*, 1992; Sun, 1992; Verkerk and Wright, 1996). Despite the diversity of crop management practices in different regions requiring different IRM tactics, research results have highlighted some common features in DBM resistance in Southeast Asia:

- DBM can develop high field resistance to new insecticides quickly (average of two years after market introduction).
- Resistance is unstable in most cases; reversion can be as rapid as development when insecticide selection pressure is relaxed (Noppun *et al.*, 1984; Sinchaisri *et al.*, 1989; Fahmy and Miyata, 1992; Hama *et al*., 1992; Kuwahara et al., 1995).

A first step to IRM is to exploit the unstable character of DBM resistance by avoiding the continuous selection pressure with one compound. Alternation of products of different modes of action is one classical IRM recommendation, which may be followed by or used in conjunction with other well-known methods of IRM.

IRM-IPM holism

Although farmers have traditionally controlled pests by judicious use of direct or indirect methods, the concept of integrated pest management was scientifically formalised and promoted in the 1950's and 60's as a result of problems caused in part by

excessive use of synthetic pesticides. We evoke the original definition of IPM by R.F. Smith and Reynolds (1966) – "IPM is a pest management system that utilises all suitable techniques in a compatible manner to reduce pest populations and maintain them at levels below those causing economic injury". One simplistic but basic rule of IPM is *that suitable pesticide types should be used correctly and only when necessary*. This prevents unnecessary pest selection pressure. Considering IRM, the basic principle is *'the preservation of pest susceptibility'*, implying that pesticides should be used only when needed. As a consequence, natural enemies of pests are spared. IRM and IPM are mutually complementary and justify the definition that IRM is an integral part of IPM as has also been mentioned by others (Phillips *et al.*, 1989; Denholm and Rowland 1992). A true IPM must integrate IRM in a holistic outlook. Unfortunately, to this date many researchers, regulators and advisers still inadvertently or intentionally overlook IRM in favour of purist IPM in their general thinking. The interpretation of IPM philosophy has been distorted by many to think of pesticide-free practice rather than the rational integration of all methods of pest management. Researchers in IPM have an obligation to take an holistic approach in every step of investigations (Lim, 1992) in order to formulate advice that has a high chance of being accepted by farmers. A holistic approach to pest management is of prime importance and encompasses more than one pest or one crop in space and time. IPM for crucifers cannot progress without IRM and vice versa. By the same token, DBM should not be considered in isolation whereas crucifers are highly attractive to a number of pest species that may occur simultaneously. Field success on a practical scale of IRM/IPM for crucifers depends on well thought out methods and implementation process.

IRM implementation

The chemical industry's contributions to IPM/IRM include basic research into finding compounds with new modes of action to avoid cross resistance, are specific to target pests, and have minimal undesirable side effects on beneficial arthropods and the environment to suit IPM requirements. However, new compounds that fulfil these requirements are difficult to find, take many years to develop and cost hundreds of millions of dollars. Beside this long term approach, industry works to promote a rational use of existing products for the sake of IRM.

The Insecticide Resistance Action Committee (IRAC) was created in 1984 under the patronage of the International Group of National Associations of Manufacturers of Agrochemical Products (GIFAP). The aim was to co-ordinate, encourage and support financially, insecticide resistance monitoring and management world-wide (Voss, 1988). One of the many IRAC grants to finance resistance research in major pests is the collaborative project conducted by Dr. E. Y. Cheng of Taiwan Agricultural Research Institute (TARI) on the pattern of DBM resistance to benzoylureas (Cheng, 1993 and 1996). Based on Dr. Cheng's findings and on literature the IRAC Field Crops and Vegetables Working Group has published a one-page IRM strategy for DBM (Appendix 1, Harris, 1995). Verkerk (1995) has proposed similar IRM for DBM in Cameron Highlands giving details of a specific product alternation programme. All countries in the Pacific Rim have ongoing IPM/IRM programmes for crucifers including DBM. For example, Indonesia (National IPM program, BALISTA), Malaysia (MARDI), Philippines (National Crop Protection Center, University of the Philippines, PhilRice, CABI, funded by FAO, IRRI, ADB, USAID, etc.), Taiwan (TARI, AVRDC), Thailand (co-operation between the Department of Agriculture, Kasetsart University, Nagoya University and Japan Society for the Promotion of Science), and others. Agrochemical companies as well as the NGOs have been testing their individual IPM/IRM programmes wherever conditions allow. Additionally, there is the Asian Vegetables Network (AVNET) sponsored by AVRDC.

As one example of an IRM programme, in the TARI project Cheng *(personal communication)* used farmers themselves as experimental parameters as well as method promoter. His success relies on background knowledge of resistance and cross-resistance pattern, and on the subtle surveillance of farmers' action during the growing season. Participating farmers were given a complete package of chemicals required to protect their cabbages from all pests. Clear recommendations for product usage (including components of mixtures if essentially needed) on different pests were also given. Co-ordination, on-site training and discussion and record keeping were done by farmer associations. At season's end all participating farmers gave oral reports in organised public meetings. Fellow farmers were thus referees, forcing everyone to report the real truth of his season's achievements. Discrete monitoring of insecticide usage was done by periodic residue analyses. Non compliance of recommended guidelines was penalised by exclusion from the programme the following year.

Considering the resources that are being invested on DBM management, one would expect that farmers would have no problem with the pest. Yet we still hear rumours from Thailand and Malaysia that some products introduced recently have already lost some of their initial efficacy. Whether rumours are the result of real resistance development or not, they are early warning signs of the well-known recurring problem.

The IPM/IRM programmes mentioned above are in the experimental stage and sometimes methods are complicated for farmers to follow. More often than not, the real interest and habits of farmers and pesticide retailers are not foremost in the minds of researchers, politicians and international financial donor organisations. Shortcomings of human nature in research and implementation of IPM have been discussed by Lim (1990). Literature on IPM tends to blame the pesticide industry (for paying lip-service)

and insecticides (for killing natural enemies) for failures of IPM. Such an attitude is consistent with Lim's remark – the authors' motivation is driven by self-interest. To paraphrase Lim – **unless we all can awaken to this sub-conscious weakness and are willing to turn around – we will not see crucifer growers practising IPM/IRM in the not too distant future;** DBM will then be the sure winner. Current scientific and technical knowledge is sufficient to go beyond publishing scientific papers and reach the farmers at all levels and all time.

On behalf of IRAC we challenge the academia, governments, international aid organisations and NGOs to join forces in bringing together farmers and pesticide dealers for discussions, education and implementation of IPM/IRM methods that are relevant to the farmers' needs. Discussions on IPM/IRM recommendations, scientific or political, should include the pesticide industry; this has rarely been the case to date. There have been bilateral co-operations at scientist level such as the IRAC-TARI project or individual company-research institute collaborations, but industry is still regarded with suspicion by some individuals in various organisations and NGOs. Whether we like it or not, in rational agricultural management pesticides will always be available to farmers when needed. Industry offers itself as a competent partner for dialogue and co-operation with all bodies concerned with agricultural production and pest management, be it in research or in training of farmers and pesticide retailers. Mutual suspicion and indirect squabble are sterile and only cause wastage of resources and valuable time for managing DBM resistance. Reputable pesticide manufacturers are strictly controlled by their own internal scientific and ethical standards as well as by governments, closely refereed by international organisations (e.g. FAO, WHO) and the media. They have a vested interest in maintaining their reputation, the quality standard of their products and the desire for their products to be used properly and intelligently. The obvious reasons are that pesticides are valuable tools that help protect crop losses from pest damages, but require huge resources for their discovery and development. Prolonging the effective life of products through appropriate IRM will benefit all (producers, users and consumers alike). IRM will make a major contribution to sustainability in agriculture.

Concluding remarks

Insecticide resistance in DBM remains the most difficult, but not intractable, problem to manage in cruciferous crops.

The recurring problem of DBM resistance to new insecticides is caused by the insect's ability to use more than one mechanism of resistance. The problem is further aggravated by the farmer's habit of using a single product continuously until it becomes ineffective.

Significant knowledge has been accumulated by researchers during the past decade, but IPM/IRM implementation at farmer's level is still at embryonic stage. Close multilateral co-operation, especially between the public and private sectors must be strengthened further if there is genuine will to help farmers and pesticide retailers.

IRAC offers itself as a competent partner in implementing IRM and is challenging the public sector (international organisations, governments and NGOs) to candid dialogues and closer co-operation as a way forward to rational pest management for the real benefit of all.

Farmers and pesticide retailers must be closely involved in the development and training of IRM/IPM methods.

Acknowledgements

We thank the workshop organising committee for giving us the opportunity to present the industry view. Thanks are also due to researchers who have co-operated with IRAC or with individual companies and to all who have contributed to the knowledge of DBM resistance.

References

Abro, G. H., Dybas, R. A., St. J. Green, A., Wright, D.J. (1988). Toxicity of avermectin B1 against a susceptible laboratory strain and an insecticide-resistant strain of *Plutella xylostella* (Lepidoptera: Plutellidae). *Journal of Economic Entomology* ***81:*** 1 575–1 580.

Adachi, T. and Futai, K. (1992). Changes in insecticide susceptibility of the diamondback moth in Hyogo, Japan. *Japan Agricultural Research Quarterly* ***26(2):*** 144–151.

Cheng, E. Y., Kao, C-H., and Chiu, C-S. (1992). Resistance, cross-resistance and chemical control of diamondback moth in Taiwan: recent developments. In *Diamondback Moth and Other Crucifer Pests* (ed. N. Talekar) pp. 419–426. Tainan: AVRDC.

Cheng, E. Y. (1993). Collaboration on the research of BPU-type IGR-resistance in the diamondback moth, *Plutella xylostella. Report for IRAC Field Crops and Vegetables Working Group*, TARI, Taiwan, 33 pages.

—— (1994). Personal communication. Pesticide Research Laboratory, TARI, Taiwan, ROC.

Cheng, E. Y. and Kao, C-H. (1996). IRAC method No. 7. A field monitoring system for IGR resistance in the diamondback moth. *The MACA International Pesticides Conference 'Crop Protection Toward 2000'*, Kuala Lumpur, 1996.

Denholm, I. and Rowland, M. W. (1992). Tactics for managing pesticide resistance in arthropods: Theory and practice. *Annual Review of Entomology* ***37:*** 91–112.

Fahmy, A. R. and Miyata, T. (1992). Reversion of chlorfluazuron resistance after release from selection pressure in the diamondback moth, *Plutella xylostella. Journal of Pesticide Science* ***17*****:** 83–85.

Furlong, M. J. and Wright, D. J. (1994). Examination of stability of resistance and cross-resistance patterns to acylurea insect growth regulators in field populations of the diamondback moth, *Plutella xylostella*, from Malaysia. *Pesticide Science* ***42:*** 315–326.

Hama, H., Suzuki, K. and Tanaka, H. (1992). Inheritance and stability of resistance to *Bacillus thuringiensis* formulations of the diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Yponomeutidae). *Applied Entomology and Zoology* ***27(3):*** 355–362.

Harris, J. G. (1995). The efficacy of different strains of *Bacillus thuringiensis* for diamondback moth control in South East Asia, and their strategic usage to combat resistance to chemical insecticides, especially acylurea compounds. In *Bacillus thuringiensis Biotechnology and Environmental Benefits* (eds.Feng, T-Y, et al.), vol. 1, pp 259–268.

Kuwahara, M., Keinmeesuke, P. and Sinchaisri, N. (1995). Present status of resistance of the diamondback moth, *Plutella xylostella* L., to insecticides in Thailand. *Applied Entomology and Zoology* ***30(4):*** 557–566.

Lim, G. S. (1990). Development and implementation of integrated pest management in the developing tropics. *Proceedings Third International Conference on Plant Protection in the Tropics*, vol. 4, pp 136–144.

Lim, G. S. (1992). Integrated pest management of diamondback moth: practical realities. In *Diamondback Moth and Other Crucifer Pests* (ed. N. Talekar) pp. 419–426. Tainan: AVRDC.

Martinez-Ramirez, A. C., Escriche, B., Real, M. D., Silva, F. J. and Ferre (1995). Inheritance of resistance to a *Bacillus thuringiensis* toxin in a field population of diamondback moth (*Plutella xylostella*). *Pesticide Science* ***43:*** 115–120.

Murai, T., Miyaki, M. and Ozuka, M. (1992). Changes in insecticide susceptibility of the diamondback moth in Shimane, Japan. *Japan Agricultural Research Quarterly* ***26(2):*** 152–156.

Noppun, V., Miyata, T. and Saito, T. (1984). Decrease in insecticide resistance in the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) on release from selection pressure. *Applied Entomology and Zoology* ***19(4):*** 531–533.

Phillips, J. R., Gravers, J. B. and Luttrell, R.G. (1989). Insecticide resistance management: relationship to integrated pest management. *Pesticide Science* ***27:*** 459–464.

Shelton, A. M., Robertson, J. L., Tang, J. D., Perez, C., Eigenbrode, S. D., Priesler, H.K., Wilsey, W.T. and Cooley, R. J. (1993). Resistance of diamondback moth (Lepidoptera: Plutellidae) to *Bacillus thuringiensis* subspecies in the field. *Journal of Economic Entomology* ***86(3):*** 697–705.

Sinchaisri, N., Chomkwa, S., Saito, T. and Miyata, T. (1989). Increasing of susceptibility of insect growth regulators of R-strain diamondback moth, *Plutella xylostella* L. *The First Asia-Pacific Conference of Entomology, Chiang Mai, Thailand,* Abstract vol., p 231.

Smith, R. F. and Reynolds, H. T. (1966). Principles, definitions and scope of integrated pest control. *Proceedings of FAO Symposium on Integrated Pest Control, Rome 1965*, ***1:*** 11–17.

Sun, C. N. (1992). Insecticide resistance in diamondback moth. In *Diamondback Moth and Other Crucifer Pests* (ed. N. Talekar) pp. 419–426. Tainan: AVRDC.

Syed, A. R. and Loke, W. H. (1995). Development and challenges in pest management of vegetables. *Seminar on Development and Challenges in Pest Management in Malaysia*, MAPPS, March 1995.

Tabashnik, B. E. (1994). Evolution of resistance to *Bacillus thuringiensis*. *Annual Review of Entomology* ***39:*** 47–79.

Talekar, N. and Shelton, A. M. (1993). Biology, ecology, and management of the diamondback moth. *Annual Review of Entomology* ***38:*** 275–301.

Tang, J. D., Shelton, A. M., Roush, R. T. and Moar, W.J. (1995). Consequences of shared toxins in strains of *Bacillus thuringiensis* for resistance in diamondback moth. *Resistant Pest Management* ***7(1):*** 5–7.

Tang, J. D., Shelton, A. M. and Roush, R. T. (1995). Stable resistance to *Bacillus thuringiensis* in *Plutella xylostella*. *Resistant Pest Management* ***7(1):*** 8–9.

UK, S., Jaruek, S. and Agus, T. (1995). Resistance management of *Plutella xylostella* L. on crucifers in Southeast Asia: Aspects of implementation. *Resistant Pest Management* ***7(2):*** 24–28.

Verkerk, R. H. J, Syed, A. R. and Wright, D. J. (1996). The development of an insecticide resistance management programme for the diamondback moth in Malaysia. *The MACA International Pesticides Conference 'Crop Protection Toward 2000'*, Kuala Lumpur, 1996.

Verkerk, R. H. J. and Wright, D. J. (1996). Multitrophic interactions and management of the diamondback moth: a review. *Bulletin of Entomological Research* ***86:*** 205–216.

Voss, G. (1988). Insecticide/acaricide resistance: industry's efforts and plans to cope. *Pesticide Science* ***23:*** 149–156.

Wearing, C. H. (1988). Evaluating the IPM implementation process. *Annual Review of Entomology* ***33:*** 17–38.

Zhu, G.R., Zhao, J. Z., Wu, S. C. and Wu, S. X. (1991). Insecticide resistance and management of diamondback moth and imported cabbage worm in the People's Republic of China. *Resistant Pest Management* ***3(2):*** 25–26.

Appendix 1

Insecticide Resistance Management Strategy for *Plutella xylostella* L. (Diamondback moth) (Prepared by IRAC Field Crops & Vegetables Working Group)[1]

Benzoylurea compounds have been used widely on cabbages because of their efficacy on lepidopterous pests and their low mammalian toxicity. The diamondback moth (DBM) in Southeast and Northeast Asia has developed widespread resistance to organochlorines, organophosphates and pyrethroids in the 1960's and 1970's and to the early benzoylureas introduced into the region in the 1980's. The risk of cross-resistance to new benzoylureas is high.

The pesticide industry, through IRAC, wishes to implement an insecticide resistance management (IRM) for the DBM.

IRM aims at preserving the pest susceptibility to insecticides by minimising the continuous selection pressure that a given product or class of compounds may exert on it. Regular monitoring of susceptibility/resistance levels is the basis for managing resistance or introducing new products.

Since there are more benzoylureas recently introduced as new products for DBM control, the IRM recommendations should address two areas:

– Countries (and/or areas) where benzoylureas are still effective,
– Countries (and/or areas) where benzoylureas are no longer effective.

Although it refers mainly to benzoylureas, this strategy should also apply to all classes of products.

1. Benzoylurea effective areas

- Do not use products of the same chemical class more than once per DBM generation.
- Limit the benzoylurea applications to two per crop cycle at maximum (never use it alone continuously).
- Always alternate a benzoylurea with other effective products of different modes of action, e.g., Bt., thiourea, avermectin, pyrrole, and/or effective organophosphate.
- Use the most effective products or mixture of products during the early crop growth stage.
- Use three or more different product groups if several sprays are needed, particularly when aimed at different pests that may occur concurrently with DBM.
- If resistance to a class of products has been confirmed locally, do not include that class in the spray programme.
- Promote IRAC strategy through group companies, local research institutes, government extension, farmer co-operatives, and farmer meetings or trainings.

2. Benzoylureas ineffective areas

- Use non- benzoylurea classes of chemicals that are effective and alternate between them. In case of control failure, change class of insecticides.
- Check possible cross–resistance to any new benzoylurea.
- Refrain from introducing a new benzoylurea into the area where cross-resistance is confirmed.
- Wait until reversion to susceptibility before attempting to introduce a benzoylurea.
- Follow the steps recommended for benzoylurea-effective areas above when introducing a new benzoylurea.
- Involve farmers, distributors, research institutes, government extension, and local co-operatives in participatory trials or demonstrations.

Notes:

– *The strategy of product rotations exploits the low biotic fitness of resistant individuals in a population, so that their frequency would decline when a different insecticide is used.*
– *The DBM could eventually develop resistance to an insecticide mixture if used long enough. Therefore, a mixture of products if judged necessary, should also be alternated with other effective products of different modes of action. In other words, appropriate product alternation is a better option than just mixture.*

[1]GIFAP asbl, avenue Albert Lancaster 79a, 1180 Bruxelles, Belgium
Tel: (02) 375 68 60 – Tlx: 62120 gifap b – Fax: (02) 375 27 93

A decade of integrated pest management (IPM) in brassica vegetable crops – the role of farmer participation in its development in Southern Queensland, Australia

S. Heisswolf[1], B.J. Houlding[2], and P.L. Deuter[1]

[1]*Department of Primary Industries Queensland, PO Box 245, Gatton QLD 4343, Australia*

[2]*Cooperative Research Centre for Tropical Pest Management, PO Box 245, Gatton QLD 4343, Australia.*

Abstract

In the mid 1980's, management of Diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), in brassica vegetable crops was at crisis level with spray failures prevalent across the industry in Southern Queensland. Insecticide resistance to synthetic pyrethroids was identified in 1986, and resistance monitoring between 1988 and 1992 showed that problems also existed with carbamates, organochlorines and some organophosphates.

A resistance management strategy was implemented in 1988 with the widespread support of industry. This strategy resulted in a summer production break, improved spray application, an understanding of resistance and the need for insecticide rotation on farm. Development work with *Bacillus thuringiensis* (Bt), crop scouting, shelterbelts and natural enemies of pests expanded the emerging integrated pest management (IPM) system over the next few years.

A joint project between Australia and China to improve IPM in brassica vegetable crops commenced in 1995. The aim of the project is twofold; to expand the tools base available to growers by investigating critical areas within the IPM system (pest forecasting, monitoring, action thresholds, spray application, natural enemies, decision aids); and to continue industry participation in the development of practical IPM systems, using action learning and adult education principles.

This paper gives an overview of the research, development and extension work with pest management in brassica vegetable crops in Southern Queensland over the last ten years. In particular it discusses the evolution of Integrated Pest Management (IPM) systems in the region and examines the changing role of farmers in developing these systems for managing and avoiding insecticide resistance in *P. xylostella*.

Key words: IPM, implementation, brassicas, Australia

Introduction

The brassica industry of Queensland is worth Aus$24 million, which is 19% of the Australian industry. Eighty percent of the crop is sold on the domestic market, with the remainder exported to Hong Kong, Singapore and Japan (Australian Bureau of Statistics, 1993). The major brassica vegetable crops grown are cabbages, cauliflower and broccoli with Brussels sprouts, Chinese cabbage and other Chinese leafy vegetables being minor crops. A total of 2 300 hectares of brassicas are produced per annum (Australian Bureau of Statistics, 1993). The average property size is 50 hectares.

The major production region is the Lockyer Valley, a river system about 100 km inland. Planting of crops begins in February (late summer) and weekly or fortnightly plantings are made until early spring (September) with last harvests in late spring (end of October). Summer production occurs at higher altitudes further inland in the Granite Belt region. Limited areas of brassica vegetable crops are also grown near the coast, north and south of Brisbane.

A suite of lepidopterous pests attack brassica vegetable crops in Queensland. These include the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), cabbage white butterfly, *Pieris rapae* (Linnaeus) (Lepidoptera: Pieridae), centre grub, *Hellula hydralis* (Guenee) (Lepidoptera: Pyralidae), cabbage cluster caterpillar, *Crocidolomia pavonana* (Fabricius) (Lepidoptera: Pyralidae), cluster caterpillar, *Spodoptera litura* (F.) (Lepidoptera: Noctuidae), and *Helicoverpa* spp. (Lepidoptera: Noctuidae). Aphids can be pests of seedlings or a contaminant of produce but generally do not cause severe damage to the crop.

P. xylostella is the most difficult pest to manage, largely due to its resistance to a range of commonly used insecticides, however *C. binotalis* and *H. hydralis* have the potential to cause more significant damage if not controlled in early season crops.

Traditionally, brassica vegetables were grown year round in the Lockyer Valley and growers schedule sprayed for pests once per week throughout the production season. Since the mid 1980s when *P. xylostella* resistance to pyrethroids was first reported (Wilcox, 1986) and resistance in carbamate, organophosphate and organochlorine insecticides was identified (Hargreaves, 1994, pers comm.), Queensland growers have gradually included pest monitoring, *Bacillus thuringiensis* var. *kurstaki* (Btk),

a production break, rotation of insecticides and improved spray application in their pest management regime.

Several factors facilitated this change in pest management practice. Spray failures and crop ploughouts were the trigger for developing and implementing alternative methods of pest management as scheduled applications of insecticides were no longer reliable. Up until the late 1980's, growers were largely interested in research focussed on efficacy of insecticides, however the control crisis mobilised industry and government to search for alternative approaches to DBM management and all sectors of the industry participated in implementation. The opportunity was also taken to develop an alternative approach to research and extension which did not rely on the transfer of technology extension model (Chambers and Jiggins, 1987). Continued participation by all sectors of the industry was seen as a key to successful implementation of research and development results within the farming system and extension activities were structured using the concepts of action learning (Kolb, 1984; McGill and Beaty, 1992; Heisswolf, 1995) and adult education (Brookfield, 1986; Tennant, 1991).

The progression of IPM in brassicas in Queensland can be divided into three discrete but overlapping projects.

- *1988 to 1990* – Development and implementation of an insecticide resistance management strategy based on rotation of insecticide groups by exclusion. Techniques which developed from this strategy included a production break, pest monitoring and improved spray application.
- *1990 to 1995* – A project designed to reduce reliance on conventional insecticides by focussing on the cropping systems level of pest management and introducing Btk into the developing IPM system.
- *1995 to 1998* – This project builds on the existing IPM system and includes research, development and extension components.

Materials and Methods

Resistance Management Strategy (1988 to 1990)

The initial response to the crisis caused by insecticide resistance in *P. xylostella* was the development of a resistance management strategy based on rotation of insecticide groups. It was used in three valleys in South East Queensland, hence its name, the 3 Valley Strategy. The strategy was launched in August 1988. It was developed by a small group of people from various organisations and the concept for the strategy received widespread support from all sections of the industry including local growers, agrichemical companies, pesticide resellers and crop consultants. Its primary objective was to reduce the rate of increase of insecticide resistance in *P. xylostella* and *Helicoverpa* sp. by asking growers to voluntarily exclude a particular insecticide group from use in particular months of the year. The strategy was aimed at all primary producers in the region (Deuter, 1989).

A logo for the strategy was developed and pesticide resellers were asked to colour-code insecticide containers according to insecticide groups. A series of articles was published in the local media and extensive publicity about the strategy was maintained for two years after its launch. (Deuter and Twine, 1988; Deuter, 1989). Growers meetings and insect identification workshops were held to encourage grower adoption of the strategy (Deuter, 1989). Operating costs for activities were shared by industry and organisations involved in promoting the strategy.

A survey to determine the level of adoption of the strategy was conducted in 1990. Data was gathered using a questionnaire to interview four groups of people; broccoli growers, local pesticide resellers, crop consultants and company field officers (Heisswolf, 1992).

Reducing reliance on conventional pesticides (1990 to 1995)

The second project aimed to further reduce reliance on conventional insecticides by developing alternative pest management techniques within an IPM framework and focussing on the cropping systems level rather than the individual pest level. Research and extension activities involved a series of demonstration plantings at a local research station and on commercial farms in the Lockyer Valley. This work was funded by the vegetable industry, state and federal governments. Unsprayed plantings of brassica vegetables were also established to collect data on the abundance of pests and beneficials over three seasons and insecticide resistance levels in *P. xylostella* continued to be monitored.

Thirty-six demonstration plantings were established between 1990 and 1993 at the local research station and over 50 commercial brassica plantings were assessed on farm from 1992 to 1995. Data collected included pest activity, yields and quality of harvested product. Results were used to recommend improvements to the farmer's pest management regime with particular emphasis on spray decision making. Apart from the intensive work with grower cooperators, farm walks and field days were used to demonstrate results in commercial crops. Numerous articles were also published in local media and industry publications describing project progress and recommendations from this work.

Improving IPM in brassicas (1995–1998)

The third project aims to build on the existing IPM system. It includes a research and development component which focuses on problem areas such as insecticide spray coverage, pest monitoring protocols, action thresholds, natural enemies particularly parasitoids, and development of decision-making tools. The project also includes an implementation component with emphasis on extension methodologies useful to IPM implementation.

The project commenced in July 1995. It is a collaborative research and development project between Queensland and China funded by the Australian Centre for International Agricultural Research (ACIAR). Objectives for the Queensland component of the project evolved from a problem definition workshop (Deuter and White, 1995) held by the Cooperative Research Centre for Tropical Pest Management (CTPM) at which farmers, consultants, scientists, extension staff, industry, chemical company staff were represented.

A team approach was adopted with team members holding skills in entomology, taxonomy, pesticide application methodology, extension and IPM development. The broad skills base of the team should contribute towards a systems approach to IPM in brassica vegetables. The process also encourages considerable grower contribution to the project with their views playing a significant role in determining the direction of research. Industry participation is encouraged through on farm trial work, collaborative planning and conduct of trial work and field days using adult education and action learning principles. These principles have also been used to structure six monthly review meetings of the project team.

Results

The Resistance Management Strategy

An evaluation of the 3-V strategy two years after development revealed that within the first year, 70% of broccoli growers had used the strategy; but 12 to 18 months later this implementation rate had dropped to 35%. Key factors for achieving a relatively high rate of adoption were extensive publicity and industry support. Factors leading to the decline of the strategy included a perceived inflexibility of the strategy, and a lack of continuing publicity. It is important to point out that of the 35% of farmers who continued using the strategy, most had adapted the strategy to suit their particular farming enterprise (Heisswolf, 1992).

The evaluation also showed that by 1990, farmer and industry awareness of responsible insecticide use and pest management techniques such as a break in production over summer, improved spray application, pest monitoring and strategic applications of Btk had increased (Deuter *et al*., 1992).

Reducing reliance on conventional pesticides

Monitoring of resistance of a range of insecticides from 1988 to 1992 showed that *P. xylostella* was relatively insensitive to methomyl, carbaryl and endosulfan and as a result the organochlorine and carbamate groups of insecticides were no longer recommended. An apparent stabilisation of resistance levels occurred in permethrin after the introduction of a summer break in 1990 (Heisswolf and Hargreaves, 1994).

Demonstration plantings showed that brassica vegetable production with significantly reduced input of conventional insecticides was possible using pest monitoring and Btk. This system minimised hazardous effects on natural enemies and farmers became more aware of predators and parasites in their crops. Since 1990, the use of monitoring and Btk by Southern Queensland brassica vegetable growers has increased substantially (Deuter *et al*., 1992) indicating that demonstration plantings, field days, on farm trial work, availability of decision aids and continued publicity about IPM have been successful tools for implementing new pest management practices.

By 1995, the focus had changed from scheduled spraying of conventional insecticides to a more complex system which combined a range of pest management techniques. These include:

- cultural control methods (production breaks, crop hygiene),
- crop monitoring to improve decision making,
- strategic applications of insecticides (biological and conventional),
- protection of local natural enemies by using pest specific insecticides whenever possible

Improving IPM in brassicas

The successful implementation of IPM techniques discussed above has led to a receptive environment for integrating research results from the ACIAR project into the existing pest management system. Close interaction with farmer cooperators will continue to be critical in refining IPM systems at the farm level and for highlighting specific research needs and implementation constraints. Grower cooperators are more proactive in their approach to pest management and are actively involved in assisting the ACIAR team realise project milestones.

Discussion

Much has been written in the past few years about the transfer of technology (TOT) model of extension and its limitations in implementing complex systems or concepts such as IPM. Tait (1983), Chambers and Jiggins (1988), Vanclay and Lawrence (1994) state that the traditional TOT model relies on a top down approach in which scientists develop methods and technologies which are disseminated by extension agencies for adoption by farmers; and that this extension model is inappropriate in many instances. Petty (1994) argues that participative methods of enquiry are required to develop sustainable farming practices so that local people are able to develop farming systems which suit their particular needs and conditions. A respect for knowledge, experience and expectations of farmers is considered critical for participatory research and extension (Chin *et al.*, 1992; McDonald and Glynn, 1994).

Traditionally the main barriers to adoption were considered to be farmer attitudes and lack of knowledge rather than logical decision making by farmers on the usefulness of a particular technology (Vanclay and Lawrence, 1994) but it appears that that the reasons for non adoption are more complex.

A recent survey of crop consultants in the United States indicates that lack of viable nonchemical tactics, potentially lower yields and quality, higher costs, need

for higher management skills and lack of information are the major limitations of IPM adoption (Ferguson *et al*., 1996). Tait (1983) suggests that poor marketing of research products is a contributing factor in non adoption of IPM and survey work by Wearing (1988) showed that an IPM program must be marketed and adapted to suit local conditions in an effort to compete with pesticides.

Lack of a crisis with existing pest management techniques is also listed as a major obstacle to IPM implementation by Wearing (1988) and Vanclay and Lawrence (1994) outline several additional factors which lead to resistance or reluctance to change including conflicting information, loss of flexibility, complexity and/or incompatibility with other aspects of farm management and personal objectives.

Over the past decade, our approach has been to actively encourage industry participation in combating the insecticide resistance problem. The process of developing IPM systems and appropriate extension processes has been evolutionary and highlight the importance of the issues discussed above.

The development of insecticide resistance in DBM and resultant spray failures and crop ploughouts in the late 1980's provided the necessary crisis and accelerated the search for alternatives to insecticides. Resistance management became a priority not only for government but industry and farmers who were unable to rely on their usual pest management technique; scheduled applications of insecticides. This, combined with industry and farmer involvement in developing the strategy provided a receptive environment for implementation.

The review of the strategy in 1990 highlighted the importance of marketing. Widespread support of the strategy combined with an aggressive publicity campaign placed additional pressure on farmers to consider the strategy as an appropriate part of their pest management system. As publicity and industry support for the strategy decreased, farmer usage also decreased, with lack of flexibility given as a major factor in discontinuing use of the strategy (Heisswolf, 1992).

By this time, additional techniques which evolved from the strategy were being utilised, including a summer production break, improved spray application and pest monitoring. Industry and farmers had adapted the strategy to fit their farming systems. IPM technology is not static but must be modified and improved as new management tools become available (Chin *et al.*, 1992).

The demonstration plantings and on farm trial work from 1990 to 1995 promoted the strategic use of Btk and pest monitoring under commercial conditions. From a farm management point of view, Btk use is not radically different from conventional insecticides – both are applied through spray equipment – although Btk requires higher managerial skills to achieve comparable results. Btk was in a good position to compete with conventional insecticides. A concerted marketing effort by Btk suppliers and participatory on farm trial work demonstrating the Btk/monitoring system were key elements in achieving adoption of these two techniques. With reduced input of conventional insecticides, natural enemies were seen more frequently in crops and farmers became more receptive to methods which would help protect natural enemies and further decrease insecticide use.

The participatory problem specification workshop (Deuter and White, 1995) in 1995 assisted in the development of the ACIAR project and utilised farmer and industry knowledge and experience. Farmer participation will continue through the use of adult education and action learning to plan and conduct on farm trial work and field days. This will ensure that a two way flow of information and ideas between the project team and industry occurs throughout the life of the project.

The future holds many challenges for IPM in brassica vegetables in Southern Queensland. Research and extension efforts are seen as one source of information for improving IPM systems and participatory processes will enable the project team to tap into farmer and industry experience and so remain responsive to changes in farmer needs. Participatory processes ensure ownership of problems and opportunities and provides a mechanism for contributing towards solutions.

Acknowledgments

A key to the success of IPM implementation has been the willingness of growers to contribute their time and expertise in the planning and conduct of projects. Financial support from the Department of Primary Industries Queensland, the Queensland Fruit and Vegetable Growers, the Horticultural Research and Development Corporation, the Australian Centre for International Agricultural Research and the Cooperative Research Centre of Tropical Pest Management has also been critical to the success of IPM development in the Southern Queensland brassica vegetable industry.

References

Australian bureau of statistics (1993) Agricultural Industries Financial Statistics, Australia 1992–93, cat. no. 7507.0, Canberra, Australia.

Brookfield, S. D. (1986). Understanding and Facilitating Adult Learning. Jossey-Bass Publishers, San Francisco, U.S.A.

Chambers, R. and Jiggins, J. (1987). Agricultural research for resource-poor farmers Part I: transfer-of-technology and farming systems research. *Agricultural Administration and Extension* ***27:*** 35–52.

Chin, H.; Othman, Y., Loke, W. H. and Rahman, S. A. (1992). National integrated pest management in Malaysia. In: *Integrated Pest Management in the Asia-Pacific Region* (ed. P.A.C. Ooi *et al.*) pp. 255–266. Oxon: CAB International and Asian Development Bank.

Deuter, P. L. (1989). The development of an insecticide resistance strategy for the Lockyer Valley. *Acta Horticulturae* ***247:*** 267–272

Deuter, P. L., Frank, B. R., Brough, E. and Heisswolf, S. (1992). Sustainable vegetable production systems – an action learning approach. *Proceedings of the Second World Congress on Action Learning* pp. 78–81.

Deuter, P. L. and Twine, P. (1988). The 3-V strategy – waging war on insecticide resistance. *Queensland Fruit and Vegetable News* ***59(15):*** 15–17.

Deuter, P. L. and White, G. (1995). *Workshop Report: Integrated pest management in brassica vegetable crops*. Cooperative Research Centre for Tropical Pest Management, Brisbane, Australia, 47 pp.

Ferguson, W.; Yee, J.; and Fitzner, M. (1996). Nonchemical pest and nutrient management practices: Limitations to adoption and policy options. *Journal of Sustainable Agriculture* ***7(4):*** 45–56.

Hargreaves, J. (1994). Personal Communication. Redlands Research Station, QDPI, Australia.

Heisswolf, S. (1992). Evaluation of a management strategy for reducing insecticide resistance in the cabbage moth, *Plutella xylostella*. *Working Papers of the Horticulture Extension Conference* Yeppoon, Australia, pp. 102–106.

Heisswolf, S. (1995). Learning to use Action Learning Cycles. *Rural Extension Centre Learning Projects, Volume 1*, Gatton, Australia, pp. 117.

Heisswolf, S. and Hargeaves, J. (1994). Managing diamondback moth resistance in south east Queensland. *RIRDC National Workshop: managing resistance in diamondback moth.* Melbourne, Australia, June, pp. 5–10.

Kolb, D. A. (1984). Experiential learning: experience as the source of learning and development. New Jersey: Prentice Hall.

McDonald, D. G. and Glynn, C. J. (1994). Difficulties in measuring adoption of apple IPM: a case study. *Agricultural Ecosystems and Environment* ***48:*** 219–230.

McGill, I. and Beaty, L. (1992). What is action learning and how does it work? In *Action Learning – A Practitioner's Guide* pp. 17–34. London: Kogan Page.

Petty, J. N. (1994). Alternative systems of inquiry for a sustainable agriculture. *ids bulletin* 25(2): 37–48.

Tait, E. J. (1983). Pest control decision making on brassica crops. *Advanced Applied Biology* ***8:*** 121–188.

Tennant, M. (1991). Establishing an Adult Teaching-Learning Relationship. *Australian Journal of Adult and Community Education* ***31(1):*** 281–92.

Vanclay, F. and Lawrence, G. (1994). Farmer rationality and the adoption of environmentally sound practices; a critique of the assumptions of traditional agricultural extension. *European Journal of Agricultural Education and Extension* ***1(1):*** 59–94.

Wearing, C. H. (1988). Evaluating the IPM implementation process. *Annual Review of Entomology* ***33:*** 17–38.

Wilcox, P. R. (1986). Resistance of cabbage moth, *Plutella xylostella*, to pyrethroids in the Lockyer Valley. *Graduate Diploma Study Project, University of Queensland, Gatton College.*

Insecticide resistance in diamondback moth (DBM), *Plutella xylostella* (L.): status and prospects for its management in India

A. Regupathy
Tamil Nadu Agricultural University Coimbatore – 641 003. India

Abstract

Insecticide resistance and concomittant field failure to control the diamondback moth (DBM), *Plutella xylostella* were first recorded in 1968 in Punjab. Studies carried out in different states like Tamil Nadu, Karnataka, Madhya Pradesh, Punjab, indicate that fenvalerate, quilnalphos and monocrotophos resistance are now ubiquitous in *Plutella xylostella.* In Tamil Nadu, the discriminating dose technique by vial assay is used to routinely monitor resistance in cabbage and cauliflower growing areas. Very high levels of resistance to fenvalerate (66–100%), significant OP resistance (quinalphos 45–81%; monocrotophos 33–86%) and low levels of resistance to cartap hydrochloride (18–53%) and carbosulfan (14–55%) are a feature of all regions monitored. The problems encountered in effective management of *P. xylostella* under Indian conditions are: 1. indiscriminate and over use of pesticides; 2. carry over of pest aided by staggered and repeated monocropping of cabbage and cauliflower and 3. nonavailability of effective biocontrol agents such as *Cotesia plutella* (Kurdjumov), *Diadegma semiclausum* (Horstmann), granulosis virus, etc. in sufficient quantities and high cost of B.t. formulations.

The extent of natural parasitisation of *P. xylostella* by *C. plutellae* varied from 16 to 75% in tropical plains and that of *D. semiclausum* to the extent of 80% in hill/highland areas. The occurrence of granulosis virus (GV) highly pathogenic to *P. xylostella* was observed in lab. culture. Attempts are being made to mass multiply this virus.

The following strategies are discussed: 1. create a network on resistance monitoring and management on the lines developed recently for *Helicoverpa armigera,* 2. obtaining base-line data for site specific IRM tactics and 3. developing and popularising available alternative control agents like parasitoids and the GV.

Key words: Diamondback moth, insecticide resistance, management strategies

Introduction

In India, diamondback moth (DBM) *P. xylostella* (L.) was first recorded in 1914 (Fletcher, 1914) on cruciferous vegetables. This species is now distributed all over India wherever cruicifers are grown. Though DBM infests important crucifers *viz*. cabbage, cauliflower radish, knol khol, turnip, beet root, mustard, *Brassica campestris* var. *toria* and *B. campestris* var. *sarson* (Chand and Choudhary, 1977; Dube and Chand, 1977; Jayarathnam, 1977; and Singh and Singh, 1982) and non cruciferous crops like *Amaranthus viridis* L. (Vishakantaiah and Visweswara Gowda, 1975), the pest exhibits a marked preference for cauliflower and cabbage. Perhaps these crops with fleshy and succulent leaves provide olfactory and gustatory stimuli for successful selection and development (Chand and Choudhary, 1977; Dube and Chand, 1977; and Singh and Singh, 1982). The crop loss is estimated to vary from 52 (Krishnakumar *et al.,* 1984) to 100 per cent (Calderson and Hare, 1986)

DBM as a major pest of crucifers

The reasons for DBM assuming the status of major pests of crucifers in India are :

1. Continuous cropping of susceptible crops (cabbage and cauliflower) throughout the year and monocropping (mustard and rape) in larger areas.
2. Diversity and abundance of natural enemies *(Cotesia plutellae, Diadegma semiclausum)* is reduced by redundant and free use of synthetic non-selective insecticides (monocrotophos, quinalphos etc.)
3. Greater competitive ability of the pest over its natural enemy in establishing itself in newer areas.
4. Ability to migrate longer distances.
5. Out-dated application technology resulting in inefficient targeting of sprayings against DBM.
6. High rate of multiplication. DBM has a capacity to multiply 3.18, 3.38 and 2.5 times every week on cauliflower, cabbage and mustard, respectively (Justin, 1996) and as many as 16 generations are completed per year (Jayarathnam, 1977)

Resistance Development

A number of insecticides have been evaluated and reported effective against DBM; endosulfan,

Table 1. Discriminating doses (LC99) used for assessing resistance in field population

Insecticide	LC_{99} (ug/ml) Method of assay		
	Vial	PST	Larval dip
Carbosulfan	4.0	20.0	15.0
Cartap hydrochloride	5.0	10.0	10.0
Fenvalerate	115.0	170.0	130.0
Monocrotophos	140.0	170.0	150.0
Quinalphos	3.0	10.0	10.0

Table 2. Insecticide resistance frequency of *P. xylostella* in different sampling locations of Tamil Nadu

Collection site	Fenvalerate (115 ppm)		Quinalphos (3 ppm)		Monocrotophos (140 ppm)		Cartap hydrochloride (5 ppm)		Carbosulfan (4 ppm)	
	No. tested	Survival %	No. tested	Survival %	No. tested	Survival %	No. tested	Survival %	No. tested	Survival %
Coimbatore	791	81.54	741	77.87	541	51.94	620	30.48	662	27.49
Ooty	1 233	78.83	965	61.67	897	43.70	832	42.43	864	29.86
Oddanchatram	781	85.66	702	63.11	730	58.63	593	34.40	764	29.06

fenitrothion, fenthion, dichlorvos, quinalphos, methamidophos, chlorpyriphos, phosalone, phenthoate, methylparathion, monocrotophos, sulprofos, prothiophos, carbaryl, cypermethrin, fenvalerate, permethrin, carbosulfan, and cartap hydrochloride (Chawla and Kalra, 1976; Singh *et al*., 1976; Rajamohan and Jayaraj, 1978; Krishnaiah *et al*., 1978; Regupathy and Paranjothi, 1980; Srinivasan and Krishnakumar, 1982; Shah *et al*., 1984; Chelliah and Srinivasan, 1986; Chandrasekaran *et al*., 1994; and Rabindra *et al*., 1995).

In India, the first report of DBM resistance to insecticides (DDT and parathion) was made by Varma and Sandhu (1968) in Ludhiana (Punjab). Subsequently this was confirmed by Deshmukh and Saramma (1973). They also observed the DBM resistance to ethyl parathion in Jalandhar (Punjab). Chawla and Kalra (1976) reported the extension of DBM resistance to fenitrothion and malathion. A high degree of resistance to cypermethrin, decamethrin and quinalphos was reported by Saxena *et al.*, (1989). The resistance to quinalphos was found to be stable (Chawla and Joia, 1992). The current status of DBM resistance to quinalphos (70X), fenvalerate (2700X) and cypermethrin (2880X) and cross resistance status to insecticides with different mode of action (cartap hydrochloride, diafenthiuron and flufenexuron) and had been reported by Joia and Chawla (1995) and by Joia *et al*., (1996).

Rabindra *et al*. (1995) reported that DBM populations from different parts of Tamil Nadu exhibited differential susceptibility to fenvalerate, monocrotophos, chlorpyriphos and *B.t.* (LC_{50} values : Oddanchatram population > Coimbatore population) indicating that some populations in Tamil Nadu were already on the road to resistance selection. Chandrasekaran and Regupathy (1996) fixed discriminating doses using F_{26} lab. reared population without exposure to insecticides for quinalphos, fenvalerate, carbosulfan and monocrotophos by different bioassay methods using third instar larvae *(Table 1)*.

Of the different methods, the vial residue bioassay was found to be preferred and was used for assessing the resistance levels in different DBM populations *(Table 2)*. Renuka and Regupathy (1996) took up monitoring of DBM resistance since June, 1995. The extent of resistance varied from 66.7–100.0% for fenvalerate, 45.5–92.3% for quinalphos, 32.6 to 85.7% for monocrotophos, 14.3 to 55.2% for carbosulfan and 17.9 to 52.4% for cartap hydrochloride. Oddanchatram population showed high degree of resistance to fenvalerate. Irrespective of the locations high frequency of resistance was observed to fenvalerate > quinalphos, monocrotophos > cartap hydrochloride > carbosulfan. Insecticide resistance and DBM control failures are now common in other states like Karnataka. [Veerappanavar (1996) UAS, Bangalore] and Uttar Pradesh [Raju, (1996) Banaras Hindu University, Varanasi].

Proposed IRM strategy for *P. xylostella* in India.

1. Crop scouting as a means of ensuring correct application timing to minimise the number of insecticide applications.
2. Resistance monitoring to determine the extent of the resistance problem and to monitor if the strategy is achieving any real benefit in reducing insecticide resistance.
3. Large temporal and or spatial restrictions on the use of insecticide groups with resistance risk potential.
4. Maximising efficiency of insecticide application to ensure coverage of the target eg. Primordia head formation.
5. Maximising advantage of biological and cultural methods to decrease DBM populations and these by the need for insecticide intervention.

Pest surveillance

A pest surveillance programme is in operation in the Centre for Plant Protection Studies (CPPS) in Tamil Nadu Agricultural University (TNAU) for major field crops. The scouting system has been implemented as a collaborative venture between TNAU and the State Department of Agriculture (SDA) since 1984. The concerted efforts taken by TNAU and SDA in implementing IPM, by applying more emphasis on insecticide application through economic threshold levels (ETLs) significantly reduced the number of insecticide applications applied by local farmers on cotton, rice, sugarcane etc. Such collaborative exercise between TNAU and State Department of Horticulture (SDH) is needed.

Resistance monitoring

The continuous monitoring of insecticide resistance in DBM must form an integral part of chemical control to enable the detection of resistance as early as possible so that its economic, toxicological and biological consequences may be obviated. In India, for the first time, a collaborative programme involving Natural Resources Institute (NRI), U.K., International Crop Research Institute for the Semi Arid Tropics (ICRISAT), Hyderabad, India and the Indian Council of Agricultural Research (ICAR), New Delhi, is successfully implemented to monitor seasonal changes in insecticide resistance in *H. armigera* in different regions and cropping systems in India and to develop regional insecticide resistance management strategy for *H. armigera*. Under this programme eight monitoring laboratories have been set up (Regupathy, 1995) and monitoring is done continuously since 1993.

At present the research on DBM is carried out in various states like Tamil Nadu, Karnataka, Uttar Pradesh, Madhya Pradesh, Haryana and Punjab funded by ICAR, New Delhi. However the co-ordination among scientists working on DBM is lacking. There is a need for a network programme on the lines of collaborative project on *H. armigera*. Chandrasekaran and Regupathy (1996) have established discriminating doses for commonly used insecticides on cabbage and cauliflower *(Table 1)*. The vial residue assay technique is successfully used in Tamil Nadu for continuous monitoring of DBM resistance (Renuka and Regupathy, 1996). These may be used in other laboratories to have uniformity. Based on this, a field kit has been developed and tested in adoptive trials with farmers and extension functionaries in order to make an informed choice as to the most appropriate insecticide to use at specific times in the season.

Temporal and spatial restrictions on the use of insecticides

At present the constraints identified in the implementation of temporal and spatial restrictions on the use of insecticides for the control of DBM in India are :

1. Farm holdings are small and generally literacy rate is low among users.
2 The first line contact for most farmers wanting advice on pest control is the pesticide dealer. Dealers are rarely impartial and will invariably advise farmers to pesticide even when none is required.
3. Poor participation by the chemical industry in educating the farmers.
4. Asynchrony of planting times and succession of cabbage and cauliflower crops.
5. The demand by the consumer for unblemished cabbage and cauliflower heads.

Regulation of application rate

Indiscriminate application of insecticides must be discouraged. After the application of blanket spray to protect the primordia/head formation stage, further spraying could be restricted to the number necessary to keep damage to no more than one hole an average per wrapper leaf of the cabbage. This approach is reported to be an effective alternative to reliance on regular weekly or fortnightly sprays (Srinivasan, 1984). He is of the opinion that the larval populations causing damage to either outer leaves, or to leaves about to cover the head, do not reduce marketable yield significantly.

Utilising biological control

The exploitation of biological and plant products to decrease the DBM population and thereby the number of chemical treatments and concurrent resistance risks should be considered.

Parasitoids

A large number of parasitoids have been recorded on DBM (Chelliah and Srinivasan, 1986 and Chandramohan, 1996 and the references there in). Among these, the egg parasitoids of *Trichogramma confusum* and *Trichogrammatoidea bactrae* accepted the eggs of DBM in the lab but failed to parasitise DBM in the field (Wuhrer and Hasan, 1993). The activity of a larval parasitoid *D. semiclausum* is noticed throughout year in Nilgiris with maximum parasitism (77%) during September.

C. plutellae is the predominant larval parasitoid of DBM in almost all the tracts of India (Chelliah and Srinivasan, 1986 and Srinivasan and Krishnakumar, 1982). It exhibits clear density dependent relationship with high level of parasitism ranging from 46.9% in Bangalore, 77.1% in Gujarat (Chelliah and Srinivasan, 1986) and 83.7% in Tamil Nadu (Chandramohan, 1994). In South India a high level parasitism of 68.5% by the gregarious larval pupal parasitoid *Tetrasticus sokolowskii* (Eulophidae) had been recorded in November (Cherian and Basheer, 1939). The pupal parasitoid *Diadromus (=Thyraella) collaris* which is common in Malaysia, Indonesia, New Zealand, Figi and Taiwan (2.70%) is recorded in Shillong (Chacko, 1968).

Predators

The bird yellow wagtails *(Motacilla flava)* were found to feed on DBM larvae, and the ants, *Tapinoma melanocephalum, Pheidole* spp and *Camponotus sericeus* were found to carry away DBM larvae in the field (Jayarathnam, 1977).

Pathogen

The larvae and less frequently the pupa are attacked by two entomopathogenic fungi, *Erynia* and *Zoophthora radicans.* Nagarkatti and Jayanth (1982) collected two diseased larvae affected by nuclear polyhedrosis virus (NPV) in Bangalore. Recently, Renuka and Regupathy (unpublished) observed epizootics of granulosis virus (GV) affecting field populations maintained for monitoring insecticide resistance. The virulence of different isolates are being assessed (Rabindra *et al*., 1996). The effectiveness of *Bacillus thuringiensis* (Berliner) (*B.t*) formulations had been reported as early as in 1969 by Narayanan and subsequently by Chandrasekaran *et al.* (1994) and Justin (1996).

Some of the constraints identified in promoting biocontrol agents in India are indicated below with a view to take up further research.

1. Mass multiplication and speedy distribution to the large number of cabbage and cauliflower growers. The effective larval parasitoids *D. semiclausum* and *C. plutellae* are solitary endo larval parasitoids of DBM. In India 25% field parasitism by *C. plutellae* was reported in *Trichoplusia ni*. The possibility of mass multiplication of *C. plutellae* on amenable host like *Corcyra* larvae need consideration.
2. Performance of parasitoids in field has been restricted. Egg parasitoids *(T. chilonis)* are not found to have potential for field release.
3. Adaptability of parasitoids. The pest DBM has better reproductive characteristics than its parasitoids. It reproduces under extremely varied conditions. The pest could breed and develop between 10°–40°C (Hardy, 1938). The ideal temperature range for *D.semiclausum* is 15–25°C. At temperature approaching 30°C parasitism drops sharply (Chandramohan, 1994). The optimum temperature range for *C. plutellae* (Chua and Ooi, 1986; Hsu and Wang, 1971) is 20–30°C. Heavy rain will annhilate *C. plutellae* population.

Lack of information

In India, field evaluation is done only to *D. semiclausum* and *C. plutellae* under the AVRDC (Asian Vegetable Research Development Centre) – IIHR (Indian Institute of Horticultural Research, Bangalore) – TNAU IPM Programme in Western Ghat region of Tamil Nadu. The information on the early inundative release of *C. plutellae* is not available. The evaluation of the potential pupal parasitoid, *Diadromus (=Thyraella) collaris* (Ichneumonidae), has not been done. The extrinsic superiority of *C. plutellae* over *D. semiclausum* under field condition and vice versa in laboratory condition is not understood clearly.

Scope for development of resistance to B.t.k.

DBM is the most susceptible insect to *B.t.* However the continued use of *B.t.* is likely to result in the development of resistance in DBM to *B.t.,* (Hama, 1992 and Tabashnik *et al*., 1990). Recent studies by Chandrasekaran and Regupathy (1996) and Justin (1996) revealed that different DBM populations exhibited differential susceptibility to *B.t*. This indicates resistance selection to *B.t.* Genetic analysis showed that the resistance to *B.t.* products HD 1 isolate of *kurstaki* serotype is autosomal, recessive and controlled by one or few loci (Tabashnik *et al*., 1990). The lack of cross resistance to *aizawai* serotype which has additional toxins offers some hope for managing resistance to *B.t.*

The low out turn of NPV and GV and limitation in mass multiplication of DBM on which these pathogens multiplied make use of these pathogen far from practical. Any breakthrough made in mass multiplication method (cell line cultures?) may be for future consideration.

Best-Bet Packages:

On farm trials to validate a 'Best Bet' management strategy for insecticide resistance DBM are to be conducted considering the following components:

1. Avoid pre-heading sprays of insecticides to retain natural enemies.

DBM infestation at 55 days after planting has the maximum effect in reducing the yield (Krishnakumar *et al.*, 1984). However, blanket spray of insecticide is needed to protect the primordia/head formation stage.

2. Grow trap crop

Tomato, when intercropped with cabbage reduced DBM – egg laying (Vostrikov, 1915; Burandy and Raros, 1973 and Sivapragasam *et al*., 1982). The problem is that late crop stages of tomato only inhibited DBM oviposition (Srinivasan, 1984) neccessitating early planting of tomato 30 days prior to cabbage. This is not attractive to the

farmers. Srinivasan (1984) later successfully demonstrated the usefulness of mustard as trap crop. However asynchrony in planting main crop (cabbage) and trap crop (sowing mustard crop 15 days prior to cabbage planting or planting 20 days old mustard seedling at the time of cabbage plantings) is considered difficult by farmers to adopt. To make it more attractive suitable mustard variety for synchronous planting needs to be selected/developed.

3. Application of neem based formulations.

Two neem extracts *viz.*, AZT (30 mg azadirachtin/ml) and Neem-Azal (3 mg azadirachtin/ml) recorded a mortality of 50 and 90 percent, respectively, after 13 days. Though there was no morphogenetic abnormalities, there was antifeedant and repellent effect apart from ovicidal action (Verkerk and Wright, 1994).

The neem formulations (Neem-Azal and Nimbecidine) and aqueous extracts of NSKE, *V. negundo*, *T. terrestis* and *M. azdarach* reduced the fecundity, longevity and leaf area consumption of DBM. A low ovicidal action was observed in all the neem formulations (Justin 1996). The neem products could be utilised in conjunction with trap crop.

4. *Application of biopesticides like B.t.*

Spraying of *B.t.* (500g/ha) at the primordial stage is most effective. Repeat application of same serotype may be avoided.

5. *Inundative release of parasitoids*

Release of larval parasitoids *D.semiclausum* in high altitude regions and *C. plutellae* in plains commencing from 30 days after planting @ 20,000 in each of five releases at 20 days interval.

6. *Application of insecticides*

Most appropriate insecticides at the recommended rate and alternating chemical groups of insecticides. As very high level of natural parasitism of *D. semiclausum* and *C. plutellae* in field are observed less selective insecticides like quinalphos (Mani and Krishna moorthy, 1984) may be avoided at peak period of parasitism. Avoiding repeat application of insecticides of same group or with similar mode of action and having cross resistance like cartap hydrochloride, diafenthiuron and flufenexuron (Joia *et al*., 1996) will delay the insect resistance.

References

Buranday, R.P. and Raros, R.S. (1973). Effects of cabbage-tomato intercropping on the incidence and oviposition of the diamondback moth, *Plutella xylostella* (L.) *Philippines Entomology* ***2:*** 369–374.

Calderon, J.I. and Hare, C.J. (1986). Control of diamondback moth in Southeast Asia by profenofos. In: *Diamondback moth management* (Talekar, N.S. and Griggs, T.D. eds.) *Proceedings Ist. International Workshop,* 1985, AVRDC, Taiwan, PP. 289–295.

Chacko, M.J. (1968). *Macromalon* sp a parasite of *Plutella maculipennis* curt in Assam (India) *Technical Bulletin of Common wealth Institute of Biological Control.* ***19:*** 97–102.

Chand, P. and Choudhary, R. (1977). Patterns of insect plant relationships determining susceptibility of food plants in the diamondback moth, *Plutella xylostella* (L.) Curtis. Mysore. *Journal of Agricultural Sciences* ***11:*** 547–549.

Chandramohan, N. (1994). Seasonal incidence of diamondback moth, *Plutella xylostella* (L.) and its parasitoids in Nilgiris. *Journal of Biological control.* ***8:*** 77–80.

Chandramohan, N. (1996). Biological suppression of diamondback moth. In : *Summer Institute on biological suppression of pests, diseases and weeds.* Tamil Nadu Agriculture University Coimbatore, India (In press).

Chandrasekaran, J. and Regupathy, A. (1996). Insecticide resistance in diamondback moth *Plutella xylostella* (L.) in India: Discriminating dose studies. *Proceedings of the XX International Congess of Entomology.* Firenze, Italy 1996. Abstract No. 19–067.

Chandrasekaran, J. and Regupathy, A. (1996). Baseline data for field monitoring system for *Bacillus thuringiensis* resistance in Tamil Nadu (India). *Plutella xylostella* (L.) populations. *Proceedings of the third International workshop on Management of Diamondback moth and other cruciferous pests* Kuala Lumpur 1996. (in press).

Chandrasekaran, J., Regupathy, A., Bhuvaneshwari, K. and Balasubramani, V. (1994). Biological evaluation of carbosulfan and Biobit (Btk) for the control of diamond back moth *Plutella xylostella* (L.) on cabbage and cauliflower. *Pestology* ***18:*** 5–12.

Chawla, R.P. and Joia, B.S. (1992). Studies on the development of resistance in the diamondback moth, *Plutella xylostella* (L.) to quinalphos in Punjab. *Journal of Insect Science.* ***5:*** 106–108.

Chawla, R.P. and Kalra, R.L. (1976). Studies on insecticide resistance in *Plutella xylostella* Linn. *Indian Journal Plant Protection* ***6:*** 170–180.

Chelliah, S. and Srinivasan, K. (1986). Bioecology and Management of Diamondback moth in India. In *Diamondback moth Management.* (ed. Talekar, N.S. and Griggs, T.D.,) *Proceedings of Ist International Workshop 1985, AVRDC, Taiwan,* pp. 63–76.

Cherian, M.C. and Basheer, M. (1939). *Tetrastichus sokolowskii* (Family: Eulophidae) a larval parasite of *Plutella maculipennis* in South India. *Proceedings of Indian Academy of Sciences.* ***9(B):*** 87–98.

Chua, T.H. and Ooi, P.A.C. (1986). Evaluation of three parasites in the biological control of diamond back moth in the Cameron Highlands, Malaysia. *Proceedings of First International Workshop Shanhua,* Taiwan. Asian Vegetable Research and Development Centre. pp. 173–184.

Deshmukh, S.N. and Saramma, P.U. (1973). Comparative susceptibility of *Plutella maculipennis* (Curtis) collected from Ludhiana and Jalandhar districts to some insecticides. *Pesticides.* ***7(1):*** 21.

Dube, R.B. and Chand, P. (1977). Effect of food plants on the development of *Plutella xylostella* (L.) (Lepidoptera, Plutellidae). *Entomon.* ***2:*** 139–140.

Fletcher, T.B. (1914). *Some South Indian Insects*. Superintendent Government Press, Madras. 565 p.

Hama, H. (1992). Insecticide resistant characteristics of the diamondback moth. *Proceedings of the Second International Workshop,* Asian Vegetable Research and Development Centre. Taiwan, 1992. pp.453–463.

Hardy, J.E. (1938). *Plutella maculipennis* curt. Its natural and biological control in England. *Bull. Ent. Research* ***29:*** 343–372.

Hsu, S.C. and Wang, C.J. (1971). Effect of photoperiod and temperature on the development of the diamond back moth, *Plutella xylostella* L. and the parasitism of the braconid, *Apanteles plutellae* kurd. *Phytopathol. Entomol.* ***1:*** 3–36.

Jayarathnam, K. (1977). *Studies on the population dynamics of the diamondback moth, Plutella xylostella (Linnaeus) (Lepidoptera: Yponomeutidae) and crop loss due to the pest in cabbage.* Ph.D. thesis. University of Agricultural Sciences. Bangalore. 215 pp.

Joia, B.S. and Chawla, R.P. (1995). Insecticide resistance in diamondback moth. *Plutella xylostella* (L.) and its management in Punjab, India. *European Journal of Plant Pathology, XIII International Plant Protection Congress.* The Hague, The Netherlands, 1995. Abstract No. 1653.

Joia, B.S., Chawla, R.P. and Udeaan, A.S. (1996). Present insecticide use practices on cole crops in Punjab and strategies for managing multiple insecticide resistance in diamondback moth *Proceedings of the XX. International Congress of Entomology.* Firenze, Italy 1996. Abstract No. 19–106.

Justin, C.G.L. (1996). *Bacillus thuringiensis Berliner in the IPM of diamondback moth Plutella xylostella (L.). (Lepidoptera : Plutellidae) on Cauliflower,* Ph.D. Thesis, Tamil Nadu Agricultural University, Coimbatore.

Krishnaiah, K., Jagan Mohan, K. and Prasad, V.G. (1978). Control of major insect pests of cabbage by new insecticides. *Indian Journal of Plant Protection,* ***6:*** 44–47.

Krishnakumar, N.K., Srinivasan, K., Ramachander, P.R. and Suman. C.L. (1984). Optimum control strategy of cabbage pests from a chemical control trial. *Singapore Journal Pri. Ind.* (In Press). cf : Chelliah, S. and Srinivasan, 1986. Bioecology and management of diamondback moth in India. *Proceedings of the 1st international workshop,* 1985. AVRDC Taiwan pp.63–76.

Mani, M. and Krishnamoorthy, A. (1984). Toxicity of some synthetic pyrethroids and conventional insecticides to the diamondback moth parasite, *Apanteles plutellae* Kurdj. *Tropical Pest. Management,* ***30:*** 130–132.

Nagarkatti, S. and Jayanth, K.P. (1982). Population dynamics of major insect pests of cabbage and of their natural enemies in Bangalore district. (India). *Proceedings of the International Conference of Plant Protection in Tropics.* Kuala Lumpur. 1982. pp. 325–347.

Narayanan, K. (1969). *Pathogenicity of Bacillus thuringiensis Berliner to some lepidopterous pests with particular reference to Plutella maculipennis (Curtis) (Plutellidae : Lepidoptera) on cruciferous crops,* M.Sc. (Ag.) Thesis, University of Madras, India.

Narayanan, K., Jayaraj, S. and Subramanian, T.R. (1970). Control of three species of lepidopteran insects with the pathogen, *Bacillus thuringiensis* Berliner. *Madras Agricultural Journal* ***57:*** 665–673.

Rabindra, R.J. Geetha, N. Renuka S. Varadharajan, S. and Regupathy, A. (1996). Occurrence of a granulosis virus from two populations of *Plutella xylostella* in India. *Proceedings of the Third International Workshop on the Management of Diamondback moth and other crucifier pests,* 1996, Kuala Lumpur, Malaysia. (in press)

Rabindra, R.J., Justin, C.G.L. and Jayaraj, S. (1995). Relative susceptibility of two larval populations of diamondback moth *(Plutella xylostella)* to insecticides and *Bacillus thuringiensis. Indian Journal of Agricultural Science,* ***65:*** 152–153.

Rajamohan, N. and Jayaraj, S. (1978). Field efficacy of *Bacillus thuringiensis* Berliner and some other insecticides against insect pests of cabbage. *Indian Journal of Agricultural Sciences,* ***48:*** 672–675.

Raju, S.V.S. (1996). Personal communication. Banaras Hindu University, Varanasi. India.

Regupathy, A. (1995). Problems and prospects of Management of insecticide resistance in *Helicoverpa armigera* in India. In *Challenging the future* (ed. Constable, G.A. and Forrester, N.W.) pp.556–562. Brisbane, Australia: CSIRO Melbourne.

Regupathy, A. and Paranjothi, G. (1980). Evaluation of certain insecticides against diamondback moth, *Plutella xylostella* Curtis. on cauliflower seed crop. *Pesticides* ***14:*** 30–31.

Renuka, S. and Regupathy, A. (1996). Monitoring of Insecticide resistance in Diamondback moth, *Plutella xylostella* (L.) in Tamil Nadu. *Pesticide Research Journal* (In Press).

Saxena, J.D., Rai, S., Srivastava, K.M. and Sinha, S.R. (1989). Resistance in the field populations of diamondback moth to some commonly used synthetic pyrethroids. *Indian Journal of Entomology* ***51:*** 265–268.

Shah, A.H. Patel, C.B. and Patel, M.B. (1984). Field evaluation of some new insecticides for the control of diamondback moth, *Plutella xylostella* L. *Indian Journal of Entomology* ***8:*** 20–22.

Singh, S.P. and Singh, D. (1982). Influence of cruciferous host plants on the survival and development of *Plutella xylostella* L. *Journal of Punjab Agricultural University Research,* ***19:*** 100–104.

Singh, D., Verma, G.C. and Ramzan, M. (1976). A field evaluation of some new insecticides against the diamondback moth, *P. xylostella* L. *Indian Journal Plant Protection* ***4:*** 117–119.

Sivapragasam, A., Tee, S.P. and Ruwaida, M. (1982). Effects of intercropping cabbage with tomato on the incidence of *Plutella xylostella* (L.) *MAPPS Newsletter* 6: 6–7.

Srinivasan, K. (1984). *Visual damage thresholds for diamondback moth Plutella xylostella (Linnaeus) and leaf webber Crocidolomia binotalis Zeller on cabbage.* Ph.D. Thesis University of Agriculture Sciences, Bangalore. India 166 pp.

Srinivasan, K. and Krishnakumar, N.K. (1982). Pest management in cabbage. In : *Annual Report,* Indian Institute of Horticultural Research, Bangalore, India. pp. 80–81.

Tabashnik, B.E., Cushing, B.L. Finson, N. and Johnson, M.W. (1990) Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera : Plutellidae). *Journal of Economic Entomology*. ***83:*** 1 671–1 676.

Varma, A.N. and Sandhu. (1968). Chemical control of diamondback moth, *Plutella maculipennis* (Curtis). *Journal of Punjab Agricultural University Research.* ***5:*** 420–423.

Veerappanavar (1996). Personal Communication. University of Agricultural Sciences Bangalore. India.

Verkerk, Robert H.J. and Wright, Dennis, J. 1994. Biological activity of Neem Seed Kernal extracts and Synthetic Azadirachtin against larvae of *Plutella xylostella* L. *Neem Newsletter* ***111(2):*** 26–27.

Vishakantaiah, M. and Visweswara Gowda, B.L. (1975). Record of *Plutella xylostella* (Linnaeus) *(P. maculipennis Curtis)* as a new pest of *Amaranthus viridis* in Karnataka. *Current Sciences,* ***44:*** 869.

Vostrikov, P. (1915). Tomatoes as insecticides. The importance of solanaceae in the control of pests of agriculture (Husbandry on the Don.) *Novotcherkassk.* ***10:*** 9–12.

Wuhrer, B.G. and Hassan, S.A. (1993). Selection of effective species/strains of *Trichogramma* (Hymenoptera : Trichogrammatidae) to control the diamondback moth *Plutella xylostella* (Lepidoptera : Plutellidae). *Journal of Applied Entomology,* ***113:*** 80–89.

Multitrophic interactions and the diamondback moth: implications for pest management

Robert H. J. Verkerk and Denis J. Wright

Department of Biology, Imperial College at Silwood Park, Ascot, Berkshire SL5 7PY, U.K.

Abstract

There is a scarcity of studies concerning *Plutella xylostella* L. dealing with either interactions between partial plant resistance and insecticides or multitrophic interactions (with or without insecticides). Such studies are vital to allow improved understanding of possible interactions in IPM programmes and may help both to develop approaches which enhance cultural and biological controls and minimise insecticide use. Laboratory studies showing the relationship between different degrees of partial plant resistance to *P. xylostella* (in various *Brassica oleracea* var. *capitata* L. and *B. pekinensis* (Lour.) Rupr. cultivars), leaf biomass consumption, leaf surface area damage and residual toxicity of two selective insecticides are presented and reasons for consistent relationships between various factors are discussed. Insecticide toxicity to *P. xylostella* larvae on plants with partial plant resistance is shown to be less compared with insecticides applied to either more or less resistant plants. Tritrophic laboratory and field studies in Malaysia show that larvae of *P. xylostella* on different host plant species and cultivars may experience different and sometimes contrasting rates of relative survival and parasitism by the key endolarval hymenopteran parasitoids, *Diadegma semiclausum* Hellén and *Cotesia plutellae* Kurdj. Finally, the value of multitrophic studies is discussed and their potential for stimulating novel strategies is considered.

Key words: host plant, parasitoid, *Plutella xylostella*, insecticide, integrated pest management

Introduction

Plutella xylostella L. (Lepidoptera: Yponomeutidae), has been intensively studied for more than forty years, reflecting its importance as a key pest of cruciferous crops in many parts of the world. During this period, most studies concerning *P. xylostella* have focused on its biology, methods of control or resistance to insecticides (e.g., Talekar and Griggs, 1986; Talekar, 1992). Insect/plant-based research on multitrophic interactions has recently been developing rapidly, although the focus has been predominantly on non-agricultural systems (Wright and Verkerk, 1995). A recent database search revealed that less than 5% of publications involving *P. xylostella* considered tritrophic (host plant-DBM-natural enemy) interactions or interactions between two or more different categories of control method (e.g., biological, cultural, physical, chemical) (Verkerk, 1995).

This paper considers ways in which an improved understanding of multitrophic interactions involving *P. xylostella*, including interactions with selective insecticides, may potentially be used to improve levels of control with reduced input of insecticides. Such approaches are vital to help understand the multitude of interactions which may occur as a result of integrated pest management (IPM) programmes and could alleviate problems caused by insecticide resistance and resurgence which have often plagued control strategies aimed at *P. xylostella*.

Materials and Methods

All laboratory experiments were maintained in a controlled environment (20±2ºC; 65% RH, 16:8 L:D).

Intrinsic host plant resistance

Leaf biomass consumption. Groups of five, two-day-old second instar *P. xylostella* larvae (SERD 2 strain: F_{18} from the field; Serdang, Malaysia) were introduced to individual Petri dishes (5 cm) containing leaf discs (5 cm dia.) cut from glasshouse-grown *Brassica pekinensis* (Lour.) Rupr. cv. Tip Top (84 days-old), *B. oleracea* var. *capitata* L. cv. Wheelers Imperial and cv. Red Drumhead (84 and 147 days-old for both cultivars). After 120 h, when most insects were either fourth instar or prepupae, mortality was assessed, leaf disc surface area damage estimated (see *Table 1*) and the leaf discs were removed, oven-dried and weighed. Control leaf discs were treated in the same way but were not infested. Biomass consumption by surviving larvae was determined by the difference between dry weights of undamaged and damaged leaf discs for each plant group.

Interactions between plant resistance and selective insecticides

Residual toxicity/plant resistance interactions. Leaf-dip bioassays were carried out using two age-classes of two glasshouse-grown *B. oleracea* var. *capitata* cultivars (Wheelers Imperial and Red Drumhead: 84 and 147-day-old) and a field strain of *P. xylostella* (SERD 2: F_{18}; see above). Four concentrations of teflubenzuron (Nomolt®) (0.02, 0.07, 2.00 and 6.00 μg ai ml^{-1}) and *Bacillus thuringiensis* (*Bt*) Berliner subsp. *aizawai* (Florbac®) (0.04, 0.11, 0.33 and 1.00 IU mg^{-1}) were assayed. Treated leaf discs were removed after 5 days and replaced with fresh, untreated leaf discs *ad lib.* Mortality was assessed on Days 5, 9

and 12. Leaf disc damage was estimated on Day 5 according to an arbitrary scale (see Leaf Biomass, above).

Tritrophic interactions

Laboratory studies. a) *Cotesia plutellae* Kurdj. (Hymenoptera: Braconidae). Single, mated three-day-old female *C. plutellae* (laboratory culture, IACR, Rothamsted, UK) were introduced to plastic Petri dishes (5 cm dia.), each containing a moistened filter paper (Whatmans No. 1; 4.5 cm dia.), a leaf disc (4.8 cm dia.) from the selected plant group and 10 two-day-old second instar larvae (insecticide-susceptible laboratory strain, Rothamsted Experimental Station, UK). Leaf discs were cut from outer leaves selected at random from at least 6 different plants from each group. Larvae were starved for 2 h prior to being transferred to the leaf discs. Parasitoids were removed from Petri dishes after 1 h, a duration found to allow oviposition attempts in most, if not all, ten larvae. Leaf discs were replaced after four days and subsequently *ad lib*. Treatments with parasitoids were replicated 8 times. Life stages were recorded after 5, 7 and 9 days and then at daily intervals until all insects had either emerged as adults or died.

b) *Diadegma semiclausum* Hellén (Hymenoptera: Ichneumonidae). Single, mated three-day-old female *D. semiclausum* (laboratory culture, IACR, Rothamsted, UK) were introduced to plastic Petri dishes (9 cm dia.), each containing a moistened filter paper (Whatmans No. 1; 9 cm dia.), a leaf disc (8.8 cm dia.) from the selected cultivar and 20 two-day-old second instar host larvae. Each treatment was replicated four times. The method was otherwise identical to that used for *C. plutellae*.

Field study (Tanah Rata, Cameron Highlands, Malaysia). Three plots (*c.* 10 x 5 m each) within the MARDI Cameron Highlands Field Station (Tanah Rata) were transplanted with each of the *B. oleracea* var. *capitata* cvs KY Cross, Super Dragon and *B. pekinensis* cv Super Queen. The plots were adjacent to one another but were separated by 3 m, the Super Queen plot being central. Except for one application of pyrethrum during the first week following transplanting, the plots were maintained insecticide-free. From four weeks after transplanting until harvest (6 weeks later), individual cabbages (n = 10) were sampled randomly at weekly intervals from each plot and fourth instar *P. xylostella* larvae were collected and transferred to Petri dishes for rearing through in the laboratory. Sampled cabbages were marked and not re-sampled.

Results

Intrinsic host plant resistance

Leaf biomass consumption. The dry weights (biomass) of leaf discs (4.8 cm dia.) from mature (147-day-old) *B. oleracea* var. *capitata* cvs. Wheelers Imperial and Red Drumhead were significantly (p <0.05) greater than those from younger (84-day-old) plants of the same cultivars and *B. pekinensis* cv. Tip Top (also 84 days-old) *(Table 1)*. Biomass consumption by *P. xylostella* larvae (SERD 2) was greater on both ages of the two *B. oleracea* cultivars than on *B. pekinensis*, and larvae consumed five-fold more dry weight on mature cv. Red Drumhead (despite slight [6.7%] mortality after five days) compared with larvae on the younger *B. pekinensis*. The relative damage to surface area of leaf discs appeared to be inversely related to the relative rate of leaf biomass consumption.

Table 1. Relative biomass consumption compared with damage to leaf surface area after larval feeding by a field strain of *Plutella xylostella* on leaf discs for five days in the laboratory[a]

Host plant / cultivar	Plant age (days)	Initial leaf disc dry wt. ±SE (mg)	Day 5 leaf disc dry wt. ±SE (mg)	Mean wt. difference after 5 days	Relative rate of leaf biomass consumption	Relative damage to surface area of leaf disc[b]
Brassica pekinensis						
Tip Top	84	51.8 ± 2.9a (n = 12)	41.6 ± 17a (n = 12)	10.2	1	1a (n = 6)
Brassica oleracea* var. *capitata						
Wheelers Imperial	84	58.9 ± 5.3a (n = 12)	33.5 ± 8.3a (n = 6)	25.4	2.5	0.96a (n = 6)
	147	99.0 ± 6.4b (n = 8)	68.4 ± 7.2b (n = 12)	30.6	3.0	0.96a (n = 6)
RD	84	44.5 ± 2.5a (n = 6)	26.0 ± 7.5a (n = 6)	18.5	1.8	0.71b (n = 6)
	147	125.4 ± 10b (n = 8)	74.8 ± 3.9b (n = 6)	50.6	5.0	0.67b (n = 6)

[a]Within columns, means followed by a common letter are not significantly (p >0.05) different (ANOVA and pairwise *t*-tests or χ^2 tests).
TT = cv.Tip Top (mortality after 120 h = 0%); WI = cv. Wheelers Imperial (mortality after 120 h = 0%); RD = cv. Red Drumhead (mortality after 120 h = 6.7%). All plants glasshouse-grown.
[b]Surface area damage based on visual assessment according to leaf surface area damage rating scale (0 = <5%; 1 = 5–25%; 2 = 26–50%; 3 = 51–80%; 4 = 81–100%): each rating determined as a proportion of total damage (sum of six replicates: maximum = 24). Significance testing using χ^2 test with binomial errors.

Interactions between plant resistance and selective insecticides

The different plant groups caused more than eight-fold and five-fold variations in residual toxicity of teflubenzuron and *Bt* subsp. *aizawai* respectively when exposed to larvae of the *P. xylostella* field strain *(Table 2)*. With both insecticides, plant groups of intermediate ditrophic status (based on survival of control insects) contributed to the lowest insecticide toxicity, whilst apparent toxicities were significantly (p <0.05) greater with both the most and the least intrinsically susceptible plant groups (84-day-old cv. Wheelers Imperial and 147-day-old cv. Red Drumhead, respectively).

Tritrophic interactions

Laboratory studies. Percentage parasitism by *C. plutellae* was significantly (p <0.05) greater on *P. xylostella* maintained on mature *B. oleracea* cvs Wheelers Imperial and Red Drumhead than on young *B. pekinensis* cv Tip Top *(Table 3)*. In contrast, parasitism by *D. semiclausum* was greatest (p <0.05) on cv Wheelers Imperial, intermediate (p <0.05) on cv Tip Top and least (p <0.05) on cv Red Drumhead. Larval mortality was caused directly by parasitism and by resistance factors in the (partially resistant) plants; the net effect of parasitism and plant resistance factors was assessed using survival analysis *(Table 3)*.

Table 2. Logit analysis of mortality data (Day 12) and leaf damage consumption rates (Day 5) for a field strain (SERD 2) of *Plutella xylostella* with teflubenzuron (TFB) and *Bt* ssp. *aizawai* (*Bta*) in leaf-dip bioassays on two glasshouse-grown *Brassica oleracea* var. *capitata* cultivars at two ages[a]

Insecticide	Cultivar[b]	Plant age (days)	Control data: % mortality[c] (Day 12)	Control data: Mean leaf damage rating[d,f] (Day 5)	LC_{50}[e,f]	95% FL	Slope[f] ± SE	RT[g]
TFB								
	WI	84	13.3a	3.8 ± 0.2a	0.10ab	0.01–0.35	0.74 ± 0.22a	4.4
		147	20.0ab	3.7 ± 0.2a	0.44bc	0.08–1.48	0.77 ± 0.22a	1
	RD	84	20.0ab	3.7 ± 0.2a	0.20bc	0.04–0.53	0.97 ± 0.23a	2.2
		147	36.7b	2.5 ± 0.2b	0.05a	0.01–0.19	0.78 ± 0.22a	8.8
Bta								
	WI	84	as above[h]		0.11a	0.04–0.21	1.38 ± 0.39a	5.2
		147			0.57b	0.28–3.03	1.28 ± 0.39a	1
	RD[i]	84			> 1	–	–	< 1
		147			0.11 a	0.06–0.18	1.87 ± 0.42a	5.2

[a]Source: Verkerk & Wright (1996a).
[b]WI and RD = *B.oleracea* var. *capitata* cvs. Wheelers Imperial and Red Drumhead, respectively.
[c]Control mortality not used for "correction" in logit analysis in order to include plant-induced effects. Significance test: χ^2 with binomial errors (χ^2 = 26.7, 3 df, p <0.001).
[d]Based on rating scale: 0 = <5%, 1 = 5–25%, 2 = 26–50%, 3 = 51–80%, 4 = 81–100% damage to leaf disc area.
[e]Units: mg ai ml^{-1} for teflubenzuron; iu mg^{-1} for *Bt* ssp. *aizawai*.
[f]For each toxicant (or controls), values within columns followed by a common letter are not significantly (p > 0.05) different (5 insects / replicate; 6 replicates / treatment; n = 30).
[g]Relative Toxicity factor for each insecticide: toxicity (measured by LC_{50}) of specific plant/insecticide combination in relation to least toxic plant/insecticide combination.
[h]Controls shared between treatments.
[i]Inadequate responses at concentrations tested prevented logit analysis.

Table 3. Survival analysis[a] of *Plutella xylostella* (laboratory strain) maintained in the laboratory on three host plant groups following exposure to two species of endolarval parasitoid

Parasitoid species Cultivar[b] / (age in days)		Hazard function	Mean time to death (days)	% censored	% parasitism[c]
C. plutellae [shape parameter = 2.667]					
Tip Top	(42)	0.053	19.0a	34	29a
Wheelers Imperial	(121)	0.076	13.3b	8	52b
Red Drumhead	(121)	0.067	15.1c	10	44b
D. semiclausum [shape parameter = 1.946]					
Tip Top	(63)	0.062	16.1a	13	32a
Wheelers Imperial	(132)	0.070	14.3a	2	52b
Red Drumhead	(133)	0.080	12.5b	5	14c

[a]Within species, means followed by a common letter are not significantly (p > 0.05) different, based on estimated standard errors derived from GLIM best-fit analysis with Weibull error distribution and censoring.
[b]Tip Top = *B. pekinensis*; the remaining two cvs are *B. oleracea* var. *capitata*.
[c]Significance tested (p = 0.05) by ANOVA and LSD following angular transformation.

Table 4. Fate of fourth instar *Plutella xylostella* collected at weekly intervals over a six week period (12.07.95–17.08.95) from three field-grown host plant groups in Tanah Rata (Cameron Highlands, Malaysia)

	Fate[a] of field collected L4 *Plutella xylostella* (mean proportion ± SE)				
Host plant / cultivar	Parasitised by *Cotesia* spp.	Parasitised by *Diadegma* spp.	Adult	Dead	n
Brassica pekinensis cv Super Queen	0.40 ± 0.24a	0a	0.60 ± 0.25ab	0a	5
Brassica oleracea cv KY Cross	0.06 ± 0.06b	0.17 ± 0.08b	0.69 ± 0.17a	0.07 ± 0.03a	45
Brassica oleracea cv Super Dragon	0.04 ± 0.04b	0.33 ± 0.11b	0.29 ± 0.08b	0.33 ± 0.08b	24

[a]Following rearing through in the laboratory.

Including censored individuals (those alive at the experimental end-point), mean time to death of *P. xylostella* exposed to *C. plutellae* was significantly (p <0.05) less on mature *B. oleracea* cv Wheelers Imperial than on similar-aged cv Red Drumhead, which was in turn significantly (p <0.05) less than on young *B. pekinensis* cv Tip Top *(Table 3)*. Mean time to death for *D. semiclausum*-exposed *P. xylostella* was significantly (p <0.05) less on cv Red Drumhead compared with cvs Wheelers Imperial and Tip Top *(Table 3)*.

Field study (Tanah Rata, Cameron Highlands, Malaysia). The mean (± SE) number of immature (L2 to pupae) *P. xylostella* per five plants (on a single assessment, over the six week period) was 3.04 (± 1.56), 1.96 (± 1.12) and 0.75 (± 0.37) for cvs KY Cross, Super Dragon and Super Queen, respectively. Although the number of fourth instar larvae recovered on *B. pekinensis* cv Super Queen was very low (n = 5), a pattern of parasitoid-host plant preference was noted, with *C. plutellae* emerging predominantly from larvae recovered from *B. pekinensis*, rather than from either of the two *B. oleracea* cultivars *(Table 4)*. This preference appeared to be reversed for *D. semiclausum*. Mortality of larvae (presumably parasitised and unparasitised) increased through the host plant sequence cvs Super Queen, KY Cross and Super Dragon and the latter cultivar contributed to significantly (p <0.05) greater mortality of larvae (unsuccessfully or not parasitised) than the other two plant groups.

Discussion

A reciprocal relationship was apparent in laboratory studies between the relative rate of biomass consumption and the relative damage to the surface area of leaf discs *(Table 1)*. On the most resistant cultivar/age group tested (147-day-old *B. oleracea* var. *capitata* cv Red Drumhead) five times more biomass was consumed by larvae of *P. xylostella*, with only two-thirds of damage to leaf disc surface area, compared with on the most susceptible plant group (84-day-old *B. pekinensis* cv Tip Top) *(Table 1)*. This was caused by the tendency for herbivores to consume greater amounts of lower status food plants to compensate for their low nutritional content and has been reported previously in other lepidopterous larvae (e.g., Feeny, 1970; Slansky and Feeny, 1977; Price *et al*., 1980; Clancy and Price, 1987).

When insecticide toxicity was assessed in relation to these interacting processes mediated by differing degrees of partial plant resistance, the toxicity of both teflubenzuron and *Bt* subsp. *aizawai* to larvae of *P. xylostella* was lowest on plants with intermediate levels of partial plant resistance and significantly greater on both more resistant and susceptible plant groups *(Table 2)*. This "U-shaped" response between insecticide toxicity and increasing degree of plant resistance is likely to be attributed to the tendency for insects on highly susceptible plants to consume a greater surface area of leaf (and so ingest more insecticide) whereas on the most resistant plant groups they succumb to the combined effects of the insecticide and plant resistant factors (Verkerk and Wright, 1996a). There are many factors which could complicate this relationship in the field, such as the bioavailability of insecticides on different plant surfaces, the inherent susceptibility and vigour of the herbivore (including activity/burden of detoxification enzymes), rate of foliar consumption, ratio of insecticide treated *vs* untreated material ingested, insecticide dose in relation to insect body weight and pressure from natural enemies. Field studies to allow better understanding of such interactions are much needed.

Tritrophic laboratory studies with three different plant groups which could be categorised as being of low, intermediate and high host plant status (121-day-old *B. oleracea* var. *capitata* cvs Red Drumhead, Wheelers Imperial and 42-day-old *B. pekinensis* cv Tip Top, respectively) according to a range of ditrophic bionomic criteria (Verkerk and Wright, 1994a) showed significant differences in the rate of parasitism on the different plant groups *(Table 3)*. Tip Top, the plant group with best ditrophic status to *P. xylostella*, contributed to significantly (p <0.05) lower rates of parasitism by *C. plutellae* and *D. semiclausum* than the other two plant groups *(Table 3)*. The mechanism giving rise to this effect is currently being investigated in the laboratory and may be related to differential host defence and occurs even when parasitism occurs in the absence of the host plant (R. H. J. Verkerk, unpublished data). Mortality of parasitoid-exposed larvae was likely to be caused not only by parasitism of a proportion of the test insects, but also by plant

resistance factors and the combined interactions between parasitoid-induced stress and plant resistance factors. Survival analysis (Crawley, 1993) provides a way of assessing the combined impact of such complex interacting processes and showed that the intermediate plant group (cv Wheelers Imperial) gave rise to the shortest survival time in the presence of *C. plutellae*. However, when larvae were exposed to *D. semiclausum*, the most resistant plant group (cv Red Drumhead) contributed to the shortest survival time. This suggests that partial plant resistance is likely to be of benefit in integrated control programmes involving these endolarval parasitoids.

When parasitism by the same two species of endolarval parasitoid was observed in the field (Tanah Rata, Cameron Highlands, Malaysia) over almost an entire cropping period on three host plant groups (*B. oleracea* var. *capitata* cvs KY Cross and Super Dragon, and *B. pekinensis* cv Super Queen respectively) parasitism by *D. semiclausum* was absent on *B. pekinensis* and almost double (33% *vs* 17%) that on the more resistant of the two *B. oleracea* var. *capitata* cultivars (cv Super Dragon) *(Table 4)*. However, this latter difference was not significant ($p > 0.05$). In contrast, parasitism by *C. plutellae* was very low (*c.* 5%) on the two *B. oleracea* var. *capitata* cultivars, but with a significantly ($p < 0.05$) greater proportion of larvae being parasitised on *B. pekinensis*. The low abundance of *P. xylostella* on *B. pekinensis* found in Tanah Rata was also noted in two other sites in the Cameron Highlands (Mensum Valley and Boh Road) during the same year (1995) (R. H. J. Verkerk, unpublished data) and may have been associated with host plant conditioning/antixenosis (to *B. oleracea* var. *capitata*) by certain field strains of *P. xylostella*; based on these and previous studies (e.g., Verkerk and Wright, 1994a), it is unlikely to be a result of antibiosis. The contrasting trends in *C. plutellae* and *D. semiclausum* parasitism of *P. xylostella* on *B. oleracea* var. *capitata* and *B. pekinensis* were also found in field studies in the Cameron Highlands in 1994 (Verkerk and Wright, 1996b) and 1996 (Arthurs, 1996). Possible mechanisms giving rise to such effects may be related to olfactory (synomone) attraction to host plants, host plant adaptation by parasitoids or co-evolution (Verkerk & Wright, 1996b). One or more of these mechanisms may occur in addition to those giving rise to plant-induced variation in parasitism and host survival in the laboratory *(Table 3)*.

Since all field-based agricultural systems are inevitably multitrophic, it is essential to evaluate and research them within a multitrophic context. Improved understanding of the many complex and inter-related processes allows informed decisions to be made about the compatibility of different tactics within control strategies. Multitrophic research within a real-world context may also open up channels for novel approaches, such as enhancing biological control through the use of partial plant resistance or low doses of specific insecticides (see Verkerk and Wright, 1994b).

Acknowledgements

We thank M.A.R.D.I. for cooperating in our field studies in Malaysia (1994–1996). The work was conducted under MAFF licences PHF 909A/1447/45 and PHF 909A/1031/54 and was supported by the Leverhulme Trust.

References

Arthurs, S. (1996). Personal communication. Dept. Biology, Imperial College of Science, Technology & Medicine, University of London, UK.

Clancy, K. M. and Price, P. W. (1987). Rapid herbivore growth enhances enemy attack: Sublethal plant defenses remain a paradox. *Ecology* **68**: 733–737.

Crawley, M. J. (1993) *GLIM for Ecologists*, Blackwell Scientific Publications, Oxford, 379 pp.

Feeny, P. (1970). Seasonal changes in oak leaf tannins as a cause of spring feeding by winter moth caterpillars. *Ecology* **51**: 565–581.

Price, P. W., Bouton, C. E., Gross, P., McPheron, B. A., Thompson, J. N. and Weis, A. J. (1980). Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annual Review of Ecology and Systemmatics* **11**: 41–65.

Price, P. W., Westoby, M., Rice, B., Atsatt, P. R., Fritz, R. S., Thompson, J. M. and Mobley, K. (1986). Parasite mediation in ecological interactions. *Annual Review of Ecology and Systemmatics* **17**: 487–505.

Slansky, F. and Feeny, P. (1977). Stabilisation of the rate of nitrogen accumulation by larvae of the cabbage butterfly on wild and cultivated food plants. *Ecological Monographs* **47**: 209–228.

Talekar, N. S. (Ed.) (1986). *Diamondback Moth Management: Proc. First Inter. Workshop, 11–15 March 1985, Tainan, Taiwan*, Shanhua, Taiwan: Asian Vegetable Research & Development Center, 471 pp.

Talekar, N. S. and Griggs, T. D. (1992). *Diamondback Moth and Other Crucifer Pests: Proc. Second Inter. Workshop, 11–15 Dec. 1990, Tainan, Taiwan*, Taipei, Taiwan: Asian Vegetable Research & Development Center, 603 pp.

Verkerk, R. H. J. (1995). *Studies on interactions between diamondback moth, host plants, endolarval parasitoids and selective toxicants*, PhD Thesis, University of London, pp. 244.

Verkerk, R. H. J. and Wright, D. J. (1994a). Interactions between the diamondback moth, *Plutella xylostella* L. and glasshouse and outdoor-grown cabbages. *Annals of Applied Biology* **125**: 477-488.

Verkerk, R. H. J. and Wright, D. J. (1994b). The potential for induced extrinsic host plant resistance in IRM strategies targeting the diamondback moth. In: *Brighton Crop Protection Conference: Pests and Diseases - 1994*, p. 457–462. Farnham, UK: British Crop Protection Council.

Verkerk, R. H. J. and Wright, D. J. (1996a). Effects of host plant-selective insecticide interactions on larvae of *Plutella xylostella* (Lepidoptera: Yponomeutidae) in the laboratory. *Pesticide Science* **44**: 171–181.

Verkerk, R. H. J. and Wright, D. J. (1996b). Field-based studies with the diamondback moth tritrophic system in Cameron Highlands of Malaysia: implications for pest management. *International Journal of Pest Management* (in press).

Wright, D. J. and Verkerk, R. H. J. (1995). Integration of chemical and biological control systems for arthropods: evaluation in a multitrophic context. *Pesticide Science* **44**: 207–218.

Dynamism in diamondback moth IPM development: The Malaysian experience

Loke, W. H., Syed Abd. Rahman, Sivapragasam, A., Fauziah, I., Md. Jusoh, M. and Hussan, A.K.

MARDI, P.O. Box 12301, G.P.O. 50774 Kuala Lumpur

Abstract

The development of an IPM program for diamondback moth (DBM) in Malaysia comprised several distinct stages. These ranged from initial appraisal of the problem and examination of strategic options, to component identification, development and integration, and eventual evaluation of interim IPM packages.

The initial IPM packages developed in the 70s and early 80s consisted of need-based treatments when tentative economic threshold levels (ETL) were exceeded. Several ETLs were evaluated. Over the years, as additional information became available, the initial IPM packages were refined and improved. Basically, this involved modifying the adopted thresholds and incorporating the role of parasitoids in the ETL. In 1987, the thresholds were again modified, respectively, to 4 larvae/plant and 7 larvae/plant with parasitism rate of at least 40% as a sub-parameter (IPM I).

Over the last few years, two variant packages (IPM II and IPM III) which take into consideration crop phenology have been developed and evaluated. Essentially, these packages were based on the rationale that the early phase of the cabbage crop (pre-heading) was more susceptible to economic damage by DBM than the later phase and needed more protection. A total of 5 trials in 2 locations (Cameron Highlands – 3, Jalan Kebun – 2) were conducted to compare all three IPM packages. Results showed that IPM II was the most superior package of the three in terms of yield and net profits. It is also the package with the least stringent ETLs, which further reduces the pressure to spray.

In addition to refinement of the DBM IPM package based on crop phenology, improvement through the use of a composite *Plutella* Equivalent threshold was also developed. This makes the IPM package more holistic as other major pests such as *Hellula*, *Spodoptera* and *Phyllotreta* are also addressed in the decision-making process.

Besides the utilisation/encouragement of classical biological control, other biologically-based technologies such as beneficial plants, trap crop, bioactive compounds (pheromones), botanical pesticides and F_1 sterility have also been studied and considered as potential tactics for the improvement of DBM IPM.

Key words: Diamondback moth, IPM, biologically-based technologies, Malaysia

Introduction

Plutella xylostella (L.) (Lepidoptera; Yponomeutidae), the diamondback moth (DBM), was first recorded in Malaysia in 1925. By 1941, it had become widely established all over Malaysia as a serious pest of crucifers. To-date, DBM remains as the most widespread and important pest of brassicas in Malaysia (Loke *et al.*, 1992a).

Since the 1940s, pesticides have been the main method of control practised by farmers. The demand for these synthetic chemicals has been substantial and seems endless. Such overdependence on insecticides has led to several pesticide-related problems such as resistance development, hazards to non-target organisms (NTOs), environmental pollution, poisoning and residues in the harvested produce.

In the 1970s, an ecological approach was adopted to manage the DBM problem. Research was intensified in biology, ecology and control tactics, particularly biological control and/or biologically-based technologies (BBTs), to develop a more sustainable and eco-rational approach to manage the DBM problem. This paper traces the dynamic development of integrated pest management (IPM) of DBM in Malaysia over the years.

Development of DBM IPM and initial packages

The development of DBM IPM in Malaysia encompassed several distinct stages. These stages involved initial appraisal of the problem, examination of strategic options, component identification, further development of component tactics, integration and eventual evaluation of the IPM package at farm level.

Several options were investigated, including resistant varieties, cultural management techniques, novel approaches like hormonal control and use of antifeedants. However, these approaches were found to be largely exploratory in nature, and mainly confined to laboratory situations (Loke *et al.*, 1992a; Syed, 1992). Success in the suppression of DBM with the use of biological control has been reported elsewhere (Loke *et al.*, 1992a). The biological control approach, therefore, was given priority consideration in the DBM-IPM model.

With the acceptance that natural enemies or parasitoids *(Cotesia plutellae, Diadegma semiclausum, Diadromus collaris)* can play a significant role, attempts were then made to begin formulating an IPM programme for DBM that encourages or enhances the action of this core component. The initial IPM approach, in contrast with the farmers' regime of frequent and heavy doses of insecticides, consisted essentially of need-based treatment when the tentative economic threshold level (ETL) of five larvae per ten plants was exceeded. *Bacillus thuringiensis* (Bt) was applied beyond this ETL. Synthetic insecticides were only used when infestation increased to 37 larvae per ten plants. In this first IPM programme, the impact of natural enemies was not incorporated in decision-making with regard to application of insecticides, largely because of inadequate ecological data then. From the first six trials conducted in both farmers' fields and experimental plots, it was found that several of the cabbage crops managed with the IPM approach were marginally superior in terms of economic returns as compared to farmers' fields (Sivapragasam *et al.*, 1985).

Over the years, as additional information became available, the original IPM programme was modified. The modifications concerned improving decision-making with respect to action needed on treatment. Essentially, these involved refining and improving the adopted thresholds as well as incorporating the role of parasitoids. For example, initial ETLs of five larvae per ten plants and 37 larvae per ten plants were, in later trials, changed to 15 and 37. These still did not incorporate the contribution of biological control agents. Subsequently, a further change was made whereby irrespective of the infestation level of DBM, no insecticide was allowed when the DBM larvae had at least 40% parasitization level.

In 1987, the ETLs were again modified. This IPM package, viz. IPM I, consisted of a three-tiered ETL which takes in account the percentage parasitization of the DBM larvae *(Table 1)*. Results of trials in the highlands and the lowlands clearly established the superiority of IPM I over prophylactic control practised by cabbage farmers *(Table 2* and *3)*. Marketable yields were 5–60% higher and up to 6-fold increases in profits were obtained in the IPM plots. The number of insecticide applications was also significantly reduced from 7 to 9 times in prophylactic plots to a maximum of only three applications in IPM plots (Syed *et al.*, 1992).

The series of IPM trials conducted between 1987–91 showed that, in general, IPM can provide higher net revenue when compared to farmers' practice of using insecticides prophylactically without any regard for natural enemies. IPM also required fewer sprays, and yet was able to secure marketable heads. No insecticide residue was detected in the crops harvested from IPM plots.

The IPM development studies carried out so far have also shown that IPM was both promising and highly encouraging. Even without considering the intangibles, such as reduced environmental pollution, less upset of existing natural balance and sustained ecological stability, etc., the decline in insecticidal inputs alone is sufficient to favour IPM over the existing over-indulgent chemical approach of farmers. Clearly, over the long term, the ecological benefits are likely to prove highly significant.

Table 1. IPM I package for DBM Malaysia

Crop age	Economic Threshold Level (ETL)	Decision
Week 1–10	<4 DBM larvae/plant	No spray
	>4 <7, parasitisation >40%	No spray
	>4 <7, parasitisation <40%	Spray Bt
	>7	Spray synthetics

Table 2. Summary of IPM I trials in Cameron Highlands*

	IPM	Prophylactic
Cabbage yield (tonnes/ha)	29.1	26.2
Gross returns @ RM0.80/kg	23 280	20 987
Production costs (RM/ha)	8 966	9 427
Nett returns (RM/ha)	14 314	11 560
Sprays frequency (min.–max.)	0–1	7–9

* Mean of 3 trials conducted between 1989–1991.

Table 3. Summary of IPM I trials in Jalan Kebun, Kelang (Lowlands)*

	IPM	Prophylactic
Cabbage yield (tonnes/ha)	17.7	9.20
Gross returns @ RM0.80/kg	14 160	7 360
Production costs (RM/ha)	8 345	8 925
Nett returns (RM/ha)	5 815	(1 565)
Sprays frequency (min.–max.)	1–3	7–9

*Mean of 3 trials conducted between 1989–1991 (on peat soil)

Further IPM refinement and consideration of other biological tactics

Over the last few years, further work to refine IPM I was carried out. This resulted in the development and testing of two variant packages, viz. IPM II and IPM III, which take into consideration crop phenology. Essentially, these two packages were based on the rationale that the early phase of the cabbage crop (pre-heading) was more susceptible to economic damage by DBM as compared to the later phase. Thus, the earlier phase needed more protection and the ETL during this phase should be more stringent as compared to that for the later phase. Details of IPM II and IPM III are shown in *Table 4*. A total of five trials in two locations involving cabbage crops (Cameron Highlands – 3 crops; Jalan Kebun – 2 crops) were conducted to compare the performance of the three IPM packages. Results showed that IPM II was the most superior of the three packages in terms of yield and net profits. *Table 5* shows the results for the highland trials. IPM II is also the package with the least stringent ETLs. This means that the need or pressure to conduct spraying is further reduced, which

Table 4. IPM II and IPM III packages for DBM in Malaysia

Crop age	Economic Threshold Level (ETL)	Decision
A. IPM II		
Week 1–4	<4 DBM larvae/plant	No spray
	>4 <7, parasitization >40%	No spray
	>4 <7, parasitization <40%	Spray Bt
	>7	Spray synthetics
Week 5–10	<8 DBM larvae/plant	No spray
	>8 <14, parasitization >40%	No spray
	>8 <14, parasitization <40%	Spray Bt
	>14	Spray synthetics
B. IPM III		
Week 1–4	<2 DBM larvae/plant	No spray
	>2 <4, parasitization >40%	No spray
	>2 <4, parasitization <40%	Spray Bt
	>4	Spray synthetics
Week 5–10	<4 DBM larvae/plant	No spray
	>4 <7, parasitization >40%	No spray
	>4 <7, parasitization <40%	Spray Bt
	>7	Spray synthetics

Table 5. Comparison of three IPM packages to control DBM in Cameron Highlands, Malaysia (mean of 2 trials)

	IPM I	IPM II	IPM III
Total yield/plot (kg)	1 146	1 189	1 062
Profits (RM)	762	782	634

is in line with the objective of minimizing insecticide usage.

The ETLs of the above IPM packages were considered by many to be scientifically useful but not pragmatic for farmers. They are also unreliable in situations where multiple pest species infestation occurs, which is often the case in Malaysia for open-cultivated crucifers. The common pest species besides DBM are *Hellula undalis*, *Spodoptera litura* and *Phyllotreta* spp. (flea beetles). Studies were conducted to evaluate the effectiveness and practicality of using *Plutella* Equivalent (PE) for pest counts:

1 *Hellula* larva	=	4 *Plutella* larvae
1 *Spodoptera* larva (3rd/4th instar)	=	2 *Plutella* larvae
1 *Spodoptera* larva (1st/2nd instar)	=	1 *Plutella* larva
1 Flea beetle	=	1 *Plutella* larva

Results showed that the PE action thresholds are more sensitive and effective as compared to DBM action thresholds (Md. Jusoh, 1996). However, its practicability in field situations involving farmers remains to be further studied.

Besides studies looking into improving and refining ETLs, further research on classical biological control and other biologically-based control tactics were also carried out as part of the dynamic development of DBM IPM. Currently, five species of hymenopterous parasitoids are successfully reared in Malaysia. These are *Cotesia plutellae, Diadegma semiclausum, Diadromus collaris, Trichogrammatoidea bactrae fumata* and *Oomyzus sokolowski*. This core group of biological control agents together with other potential ones like nuclear polyhedrosis viruses (NPV), the entomopathogenic fungus, *Erynia radicans* and entomopathogenic nematodes S*teinernema* spp. constitute the classical biological control approach, which has been the core component tactic in the DBM IPM programme in Malaysia (Ooi *et al.*, 1990 and Loke *et al.*, 1992b).

Other biologically-based approaches to further complement classical biological control involved considering the potential use of beneficial plants, trap crops, bioactive compounds (sex pheromones), botanical pesticides and F_1 sterility. Results obtained showed that intercropping cabbage with tomato and *Crotolaria striata* reduced incidence and damage of DBM on cabbage (Loke *et al.*, 1993b). *Crotolaria* was also found to have beneficial effects on longevity of C. *plutellae*. Investigation on Indian mustard, *Brassica juncea*, as a trap crop indicated that it can be used as hedgerow in the cabbage ecosystem to provide habitat diversity and dilute pest populations on cabbage, in addition to helping to conserve natural enemies within the ecosystem (Sivapragasam and Loke, 1996). Research into the use of sex pheromones of DBM in Malaysia showed that these bioactive compounds are useful as a monitoring tool in crucifer ecosystems but not very effective and economical to apply as mass trapping and mating disruption tools (Irfan, 1996). Evaluation of neem extracts and formulations showed that fresh water extracts of neem seed kernels were effective, mainly as an antifeedant, against *Plutella* and *Hellula* (Loke *et al.*, 1990a and Loke *et al.*, 1995). The results also showed that neem possessed repellent, ovicidal and growth disrupting properties. The F_1 sterility method represent a pioneering effort for DBM control in Malaysia. Studies showed that doses between 150 and 200 Gy appeared to be suitable for inducing inherited sterility of DBM. Mating competitiveness of the irradiated males was not

reduced. A release ratio of 10 irradiated DBM : 1 feral DBM was found to be effective (Dzolkhifli and Md. Jusoh, 1996).

Conclusion

A substantial amount of time and resources have been devoted to the research and dynamic development of IPM for DBM in Malaysia. From initial efforts in developing empirical packages aimed at reducing pesticide application, endeavors have yielded improved and refined packages whereby biological control and other biologically-based technologies are accorded due emphasis and pivotal roles. For Malaysia, the cornerstone of DBM IPM can be considered as increasing reliance on the use of self-regulating, living or bio-based pest management components, as opposed to synthetic ones; of exploiting biodiversity to manage the pest, and to protect the local biodiversity which manages the pest, i.e. natural control and assisted biological control should be maximised, enhanced and relied upon to do the job whenever and wherever appropriate.

IPM of DBM should also draw from a 'technology basket' and should not be rigid and biased towards one tactic. It should be viewed as an overall 'game-plan' in which certain 'set-pieces' can be activated depending on the occasion and logistics of the situation. It is also important to acknowledge that IPM is not a perfect solution all the time and seasonal breakdowns can and do occur, but over the longer term IPM should work favourably for all involved.

References

Dzolkhifli, O. and Md. Jusoh, M. (1996). Sterile insect techniques for the diamondback moth control. Paper presented in Third Int. Workshop on The Management of Diamondback Moth and Other Crucifer Pests, 29 Oct.–1 Nov. 1996, Kuala Lumpur, Malaysia.

Irfan, U. (1996). Monitoring and control of *Plutella xylostella* (L.) with sex pheromones in Cameron Highlands. Ph.D. Thesis submitted to Universiti Kebangsaan Malaysia, Bangi, Selangor, Malaysia.

Md. Jusoh, M. (1996). *Plutella* equivalent action threshold for insect pests of crucifers. Proc. AVNET-II Final Workshop. 1–6 Sept. 1996, Bangkok, Thailand (also in this proceedings).

Loke, W.H., Heng, C.K., Azman, R., Norlaila, B. and Hanisah, A. (1990a). Studies on neem (*Azadirachta indica* Juss.) in Malaysia. Proc. 3rd Int. Conf. on Plant Protection in the Tropics. 20–23 March 1990, Genting Highlands, Pahang, Malaysia.

Loke, W.H., Dzolkhifli, O., Mohd. Rani, Y. and Sivapragasam, A. (1990b). Exploring novel techniques for DBM Management. Sem. Pengurusan *Plutella xylostella* di Malaysia: Perspektif dan Strategi. 24 Aug. 1990, Kuala Lumpur, Malaysia.

Loke, W.H., Lim, G.S., Syed, A.R., Abdul Aziz, A.M., Rani, M.Y., Md. Jusoh, M., Cheah, U.B. and Fauziah, I. (1992a). Management of Diamondback Moth in Malaysia : Development, implementation and impact. In: Diamondback Moth and Other Crucifer Pests (ed. N.S. Talekar). Asian Vegetable Research and Development Center (AVRDC), Tainan, Taiwan. pp.529–539.

Loke, W.H., Sivapragasam, A., Syed, A.R. and Rani, M.Y. (1992b). Approaches to enhance biological control with parasitoids for IPM of diamondback moth in crucifers. Workshop 9W-4, Biological Control of *Plutella*. 19th Int. Congr. Entomol. 28 June–4 July, 1992, Beijing, P.R. China.

Loke, W.H., Syed, A.R., Fauziah, I., Md. Jusoh, M., Sivapragasam, A., Yusof, O., Heng, C.K. and Hussan, A.K. (1995). IPM of crucifer pests in Malaysia. Proc. AVNET-II Midterm Workshop. 21–25 Feb. 1995, PCARRD, Los Banos, Laguna, Philippines.

Ooi, P.A.C., Sivapragasam, A., Hussan, A.K. and Lim, G.S. (1990). Advances in biological control of DBM in Malaysia. Sem. Pengurusan *Plutella xylostella* di Malaysia: Perspektif dan Strategi. 24 Aug. 1990, Kuala Lumpur, Malaysia.

Sivapragasam, A., Lim, G.S. and Ruwaida, M. (1985). Experimental trials of an integrated pest management programme for *Plutella xylostella* (l.), Proc. Sem. on Integrated Pest Management in Malaysia. 16–17 Jan. 1984, Kuala Lumpur, Malaysia.

Sivapragasam, A. and Loke, W.H. (1996). Indian Mustard as a trap crop for insect pests of cabbage. Proc. AVNET-II Final Workshop. 1–6 Sept. 1996, Bangkok, Thailand.

Syed, A.R., Loke, W.H., Sivapragasam, A., Fauziah, I., Cheah, U.B., Md. Jusoh, M., Hussan, A.K. and Yusof, O. (1992). Integrated pest management package for effective control of *Plutella xylostella* L. on cabbage in Malaysia. Proc. AVNET-I Final Workshop. 23–26 Sept. 1992, Lembang, Indonesia.

Integrated pest management of diamondback moth: The Philippine highlands' experience

Jocelyn E. Eusebio[1] and Belen Morallo-Rejesus[2]

[1]*Crops Research Division, Philippine Council for Agriculture, Forestry and Natural Resources Research and Development (PCARRD), Los Banos, Laguna, Philippines.*

[2]*Department of Entomology, College of Agriculture, VPLB, College, Laguna, Philippines*

Abstract

Through the Asian Vegetable Network (AVNET) collaborative program supported by ADB, AVRDC and PCARRD, a technology on IPM-DBM using *Diadegma semiclausum* with supplemental application of microbial and selective insecticides based on economic threshold levels accelerated the transfer of technology to other areas of the Cordillera Region.

The technology adoption reduced 61% of pesticide costs with net savings of US$503/ha per cropping season. The technology was disseminated through trainings of technicians and researchers, farmers-participated demo farms, seminars and lectures, field days, radio broadcast and technology fairs.

The success of piloting IPM-DBM technology in Atok also inspired the Department of Agriculture to sustain the activities in other crucifer areas in the highlands. The government also launched the KASAKALIKASAN or the National IPM Program which aimed to make IPM the standard approach to crop husbandry and pest management in rice, corn and vegetables. Also, 1 719 farmer graduates from FFS through the TA 2019 special project of ADB with Department of Agriculture and IIBC in conjunction with KASAKALIKASAN nurtured the parasitoid and reduced pesticide usage by 80%.

Key words: Diamondback moth, technology adoption, National IPM program, Philippines

Introduction

Crucifers production in the Philippines is concentrated in the highlands of the Cordillera. In 1994, the total area devoted to crucifers (cabbage, petchay, brocolli, mustard, cauliflower, and lettuce) was 14 965 hectares with a production of 116 068 MT. About 80% of the total area planted is devoted to cabbage production. The Philippine highlands supply the bulk of semi-temperate vegetables particularly cabbage, brocolli and cauliflower to other areas of the country. However, vegetable production in the Cordilleras is heavily dependent on pesticides and inorganic fertilizers due to occurrence of pests. One of the major pests is the diamondback moth (DBM) which was reported in 1927 (Otanes and Sison, 1927) in the highlands of Baguio, Benguet and Cavite. It was observed to be a destructive pest of crucifers in the highlands in 1960 (Barroga, 1967) and in the lowlands in 1970 (Cadapan and Gabriel, 1972). The pest can cause 65–100% yield loss by voraciously feeding on the foliage of cabbage, petchay, brocolli and other crucifers. Desperate vegetable farmers have resorted to frequent application of pesticides, using higher dosages to achieve control. They also mix and experiment with other chemicals that are more potent than the recommended brands. These widespread practices have led to the development of resistance by the DBM. These unscrupulous practices have been virtually left unnoticed until the use of cyanide against DBM in the Cordilleras became an issue. The practice not only posed and caused damage to public health, it also eventually backfired against the vegetable growers when the public discovered the outrageous practice and refrained from buying Cordillera vegetables. In July 1992, the report of cyanide-laced vegetables from the Cordilleras prompted concerned sectors to look for alternate control measures.

This development provided impetus for the Asian Vegetable Network (AVNET) project on IPM-DBM technology to take off in the highlands. This project started in 1989 with technical assistance agreement between the Asian Development Bank (ADB), Asian Vegetable Research and Development Center (AVRDC), and Philippine Council for Agriculture, Forestry and Natural Resources Research and Development (PCARRD) for a collaborative vegetable network. The project paved the way for the technology of integrated pest management of DBM to be transferred to the Philippines.

Description of IPM-DBM Technology

The technology involves the release of parasitoids *Diadegma semiclausum* (highlands) and *Cotesia plutellae* (lowlands) to control DBM. Insect release is supplemented with microbial insecticides, based on economic threshold level (ETL).

The *Diadegma* parasite effectively controlled DBM in some European countries and in New Zealand. As such, a strain of *D. semiclausum* was introduced to the Philippines by Dr. Talekar through the AVNET Program. *C. plutellae* Kurdj., on the other hand, is an indigenous parasitoid that has been recorded in the Philippines since 1982. It was initially found in Baguio and the mountainous province of Benguet. It has never

been found in the lowland areas, hence a more efficient strain of *C. plutellae* from Taiwan was imported and introduced in selected lowland areas in the country.

Technology Promotion

- *Mass Rearing of Parasitoids*

  Mass rearing facilities were established at the Benguet State University (BSU) and at the University of the Philippines, Department of Entomology, to support the intensive mass production of the parasitoids. The parasitoids were distributed for free to vegetable farmers who are interested in availing of the technology, for sustained released in various crucifer growing areas infested with DBM in the highlands and lowlands.

- *Field Releases in Pilot Areas*

  The IPM-DBM technology was transferred to the farmers through farmer-participated demo farms. The cooperators were chosen according to their willingness to adopt the technology and to refrain from using organic insecticides. Cooperators can only use biological insecticides.

  The cocoons of *D. semiclausum* housed in elevated small type 'A' release cages were placed in the middle of the cabbage fields. Parasitoid release was done at least 3–4 times for each cooperator proportionate to the number of cabbage plants. The ideal rate was 200 parasitoids for every 500 heads cabbage.

- *Monitoring of the parasitoids*

  Benchmark information was obtained prior to the initial release of *D. semiclausum* in all the release sites. Monitoring was done by sampling 100 3rd and 4th instars of DBM per 100 plants for each cooperator. Monitoring started at 40 days after transplanting repeated at three-week intervals until near harvest or about 3–4 times throughout the cropping season. DBM larvae collected from the field were cultured in the rearing house. The rate of parasitism was computed based on the number of parasitoid cocoons recovered from the total DBM larval samples.

- *Popularization and spread of technology*

The technology was also disseminated through the following modes *(Table 1)*:

a) Training of technicians and researchers

The first training course of the AVNET-IPM-DBM project was sponsored by Japan Shipbuilding Industry Foundation (JSIF) in 1991. It was conducted for one month for technicians and researchers actively involved in crucifer production. The course was designed to: a) enable the participants to understand the principles of IPM and the role of the parasitoids in the management of DBM; b) to know the techniques of mass rearing the parasitoids for field utilization; and, c) learn the technique of field releases and assessment of parasitism for DBM management.

The training curriculum includes lectures on IPM principles, chemical selectivity, development of resistance of DBM for insecticides, and actual laboratory work on mass rearing, field releases and assessment of parasitism as well as field visits to crucifer growing areas.

Table 1. Mode of technology dissemination, 1991–1994

Activity	Frequency	Participants/Clientele
1. Exhibits/Posters	12	Policy makers Scientists Researchers Technicians Students
2. Publication in scientific journal	6	Researchers Scientists Policy makers
3. Press releases/Radio/TV broadcast	9	Scientists Farmers Technicians Students
4. Flyers/Primers/Comics	more than 1,000 copies distributed	Farmers Researchers Technicians Local community leaders
5. Field days	5	Local community leaders Farmers Technicians Scientists Researchers
6. Trainings	3	Technicians Researchers Farmer-cooperators

b) Seminars and lectures
On-site seminars and lectures on the IPM-DBM technology were conducted on farmers' fields prior to the release of parasitoids. This was organised in collaboration with local government units and the Department of Agriculture (DA).

c) Field Days
During the harvesting season for cabbage, field days were conducted through cross visits of cabbage farms of cooperators to showcase the success of the parasitoid-based IPM-DBM technology.

d) Popular publications. These were published and distributed as primers, flyers and comics on IPM-DBM technology to farmers and agricultural technicians.

e) Radio broadcast and press releases
The technology was aired regularly in local broadcast radio to reach out to the local farmers in far flung areas.

f) Participation in technology fairs
The parasitoid-based IPM technology was exhibited in different technology fairs to promote the technologies to other scientists/ researchers.

g) Special hands-on training
Interested agencies requested special hands-on training on the mass rearing of parasitoids to sustain release of parasitoids in cabbage farms.

Technology Adoption

Field Releases and Efficiency of D. semiclausum

During the AVNET Phase I, a total of 144,684 *Diadegma* cocoons were released in pilot areas of 4.94 hectares of cabbage field in La Trinidad, Atok and Buguias.

In 1992, the Department of Agriculture, through the Highland Agricultural Development Program (HADP) and the local government units took the responsibility of *D. semiclausum* releases in Bauko, Kabagan, Mankayan, Buguias. The release sites of the DA-HADP were Bauko, Kabagan, Mankayan and Buguias. The DA-CAR released the parasitoids in three other towns: Tuba, Loo and Buguias.

Initial stage of field releases of *D. semiclausum* in 1991 recorded a parasitism rate of 26-37% in the February to July croppings and 26–40% in the October to July croppings.

In 1992, after two years of continuous releases of the cocoons, parasitism reached 70–100% for the January to March and April to August croppings. Parasitism was consistently high in all locations. The outstanding performance of *D. semiclausum* could be attributed to the ability of the parasitoids to adapt and breed in the area.

Dispersal of D. semiclausum from the release sites

The presence of parasitoids was noted 0.7 km away from the previous release site. It was also noted to be present in nearby cabbage areas, which implies that the parasitoid disperses in search for food when the original sources have been depleted after harvest. It was observed that parasitism ranged from 73–93% at a distance 6 km away from the release sites compared to nearby cabbage fields (24–41%). It is therefore presumed that the parasitoid could have dispersed further as indicated by very high parasitization rate even at a distance of 6 km. This is a good determining factor for distance of release points and the number of release sites.

Technology Acceleration

Inspired by the success generated by the AVNET IPM-DBM project in piloting the technology in Atok, the Department of Agriculture signed on October 14, 1992, a Special Order No. 674, creating an Interagency Program for the IPM in the highlands. The program was aimed to properly address the persistent problem of vegetable farmers as well as the cyanide scare through the implementation of the IPM Action Project for DBM. The project accelerated the transfer of technology and spearheaded intensive trainings, information campaigns, mass rearing field releases, and monitoring in the highlands. Release areas were expanded to 22 sites in Benguet and Mt. Province. Through these inter-agency efforts, the success of the techhology adoption have evolved into Memo Order No. 176, implementing the "Kasaganaan ng Sakahan at Kalikasan (KASAKALIKASAN), the National IPM program, which supercedes S.O. 674. Spearheaded by the DA, the program aims to make IPM the standard approach to crop husbandry and pest management in rice, corn and vegetables. The KASAKALIKASAN promoted the practice of IPM among farmers through training of farmers in season-long Farmers Field Schools (FFS), training of IPM trainers, and farmers training other farmers.

On May 14, 1994, TA 2019 was approved by ADB and implemented by the Department of Agriculture and IIBC. This special project worked in conjunction with the KASAKALIKASAN in the expansion and sustaining the IPM-DBM in the Cordillera highlands. Through this TA, 1,719 farmer graduates from 65 FFS introduced and nurtured the parasitoid *Diadegma* and reduced pesticide usage by 80%. Also, a total of 119,850 *Diadegma* cocoons were released in nine municipalities from 1994–1996 (TA Final Report, 1996).

Local officials who have witnessed the success of the technology fully support the IPM-FFS as the training encourages farmers to adopt environmentally-friendly and sustainable practices. This approach empowered farmers and enhanced their decision-making towards successful farming.

Potential Benefit of the Technology

A total area of 9,000 hectares are devoted to cabbage production grown each year with potential yield of 25 t/ha. The cost of pesticides per hectare is US$838, hence the total cost of pesticides needed each year to control DBM is US$7,542,000. Using the IPM-DBM

and parasitoids, the pesticide cost is reduced to US$3.020M. Therefore, the total savings from 9,000 hectares, if all farmers utilized technology would be US$4.52M. This will lessen the drain on the country's dollar reserves brought about by the importation of pesticides.

Conclusion

As a whole, the technology offers a sustainable and safe means of controlling one of Asia's most persistent insect pests. By reducing or eliminating the use of pesticides, the technology lessens the risks to human health and helps protect the environment.

References

Barroga, S. F. (1967). Screening of chemicals for the control of diamondback moth *(Plutellae xylostella)* attacking cabbage. *Auto. Rep. Bull. Plt. Ind. Fy* 1966–67. 25 pp.

Cadapan, E. P. and B. P. Gabriel. (1972). Field evaluation of Dipel and other commercial *Bacillus thuringiensis* in comparison with the standard insecticides used in the control of diamondback moth, *Plutella xylostella* L. and other insect pests attacking crucifers. UPCA-Rep. 1972–1973. 15 pp.

Eusebio, J. E. and J. L. Acedera. (eds). (1993). Asian Vegetable Network (AVNET) Phase I - ADB RETA 5517. Terminal Report. August 1989-April 1993. PCARRD, Los Banos, Laguna, Philippines. pp. 5–73

Eusebio, J. E. and J. L. Acedera. (eds). (1996). Asian Vegetable Network (AVNET) Phase III - ADB RETA 5517. Terminal Report. May 1993-August 1996. PCARRD, Los Banos, Laguna, Philippines. pp. 70–98

IIBC - Final TA Report (1996). Integrated Pest Management for Highland Vegetables. ADB 2019-PHI. 09 May 1994 to 30 Sept. 1996. 56 pp.

Morallo-Rejesus B., A.S. Sayaboc, G.P. Fajardo and J.E. Eusebio (1994). Introduction and field releases of larval parasitoid *Cotesia plutellae* (Kurdj) for the management of diamondback moth, *Plutella xylostella* (L.) of crucifers in the Philippines. *Phil. Agric. 77(4):* 481–489

Otanes, F.W. and P. Sison (1927). Notes on the diamondback moth, *Plutella maculipennis* Curtis. *Phil. Agr. Rev.* 251–254

Development and use of a biological control — IPM system for insect pests of crucifers

K. Duane Biever

Biever Consulting, 1 South 36th Avenue, Yakima, Washington, 98902, USA

Abstract

An effective biological control-integrated pest management (BC-IPM) system that maximizes biological control of *Plutella xylostella* (L.), *Artogeia rapa*e (L.), and *Trichoplusia ni* Hubner) has been developed. In the first phase, chemical insecticides were replaced by the judicious use of the microbial insecticide *Bacillus thuringiensis*; there was an immediate reduction (>50%) in the number of treatment applications required. Technology and procedures for using parasitoid species for seasonal inoculations were developed; field tests established that a complex of several species of parasitoids increased parasitism, reduced pest populations, and resulted in market acceptable cabbage and broccoli crops. The system was successfully validated in several fields of commercial cabbage in the Rio Grande Valley of Texas; a commercial insectary produced, sold, and released the beneficial insects. A commercial BC-IPM system that emphasizes augmentative releases of *Cotesia plutellae* Kurdjumov on several hundred ha of broccoli and cauliflower has been in operation in Guatemala and Mexico for the past three years. A program to manage *P. xylostella* and other crucifer pests has recently been initiated in Mauritius. The initial focus is on the development of a population database and construction of a facility for production of *P. xylostella* and *C. plutellae*. An evaluation and implementation phase will concentrate on the augmentative releases of *C. plutellae* and the judicious use of *B. thuringiensis*. A later phase of the program will utilize the combination of inherited sterility and releases of *C. plutellae* to manage DBM populations.

Key words: Diamondback moth; augmentation; parasitoids; management system

Introduction

During my career as a research scientist with the United States Department of Agriculture, Agricultural Research Service (Columbia, Missouri and Yakima, Washington, USA), we developed an alternative management system that utilizes augmentative releases of parasitoids and provides effective control of the complex of insect pests on cruciferous crops. The Biological Control-IPM System (BC-IPM) is environmentally sound and in grower use; it focuses on biological control, population monitoring and the inoculative releases of beneficial agents. The system replaces one that relies entirely on the routine application of chemical insecticides (Biever *et al.*, 1994).

It is increasingly difficult to find replacement insecticides for use in control of insect pests, thus we must develop alternate approaches to insect management. For most crop systems, research is focusing more on integrated pest management and an important component is biological control. Although biological control is often thought of, and developed as, a separate and distinct management approach, it should be the foundation of ecologically based systems of pest control which we call IPM. Regardless of the crop system, the process for developing the BC-IPM systems is similar and includes the following steps: (1) acquire fundamental biological information, (2) establish procedures for monitoring pest populations, (3) reduce the number of insecticide applications, (4) replace chemical pesticides with biological insecticides, and (5) maximize use of biological controls. Development of a BC-IPM system is usually slow and the degree of success is relative to the effort expended. The crucifer BC-IPM project was a 24 year process from initiation to commercial use and required several crisis situations to move it from the research plots to grower fields.

Bacillus thuringiensis – IPM program development

The goal of the program was to develop and implement a system which integrates a number of biological control agents and tactics into a comprehensive program to manage a complex of pests. It was the initial hypothesis that a management system that relied on the use of biological (microbial) insecticides rather than synthetic chemical insecticides would permit the survival of naturally occurring beneficial agents that, in turn would assist in reducing the pest populations. Also, we hypothesized that once a management system was established that minimized the need for synthetic chemical insecticides, we could further enhance suppression of the pests through early-season augmentative releases of selected beneficial agents. Appropriately timed, early-season, inoculative releases of beneficials should require relatively low numbers, because population densities of the pest species are usually lowest during their early generations each season, by introducing the beneficials into pest populations that are below economic levels, the beneficials should persist and increase in numbers if hosts are available throughout the growing season. Cabbage was selected as the test crop as it is an

excellent challenge for biological control because several pests attack this crop every year, numerous applications of chemicals are applied routinely, and the final product the grower harvests must be free of insect damage. The process consisted of three phases of development: (1) research to replace chemical insecticides with biological insecticides, (2) implementation of phase one, and (3) seasonal inoculative releases of beneficial agents to maximize biological control.

The initial phase replaced chemical insecticides with the biological *Bacillus thuringiensis*. The system was based on regular field observations, knowledge of the relationships between host plants and pests, and understanding when and where to apply *B. thuringiensis*. To acquire the basic information we conducted population studies at an organic farm (Ferguson, St. Louis County, Missouri) during the 1967 growing season. This farm had not been exposed to any chemical pesticides for over 17 years, and the cabbage crop (1–1.5 ha annually), usually heavily damaged by lepidopteran pests, was far below market standards. Small plots, consisting of about 1,000 cabbage plants, (0.04 ha), were planted monthly (April to September) to provide a continuous supply of plant hosts for insect populations. The study plots, along with the grower's cabbage fields, were monitored every 3 days to collect population data on the imported cabbage worm (ICW) *Artogeia rapae* (L.) (Lepidoptera:Pieridae), the diamondback moth (DBM) *Plutella xylostella* (L.) *(*Lepidoptera:Yponomeutidae*)*, the cabbage looper (CL) *Trichoplusia ni* (Hubner) and associated beneficial insects. ICW was the primary pest of spring cabbage grown at this location with populations at times exceeding 2 large larvae per plant; DBM was most abundant during the summer (over 4 per plant throughout July) and was at low levels the rest of the growing season; CL, considered the major pest of cabbage in the St. Louis area from late June through September, was not a pest at this farm during 1967 and, according to the grower never had been. Fewer than 10 CL larvae were observed throughout the entire season.

In 1967, *B. thuringiensis*, was a relatively new biological insecticide and was being used on a limited basis. We considered it to be the appropriate biological insecticide to incorporate into our management program; however, we were in need of field evaluation experience and data. After we thoroughly explained the use of *B. thuringiensis*, the grower permitted application to one of his cabbage fields. Good control was obtained with two applications of Thuricide 90 TS (5 liters/ha), one made against each of the two generations of ICW (first-instar). This produced a bumper crop and far more than his specialty market could absorb as he usually expected to lose about 50% of his crop. Insecticide resistance in CL was observed in 1967 and during 1968 and 1969 CL populations became unmanageable even though insecticides were applied every other day. Populations of large CL larvae reached 10–25 per plant and naturally occurring epizootics of nuclear polyhedrosis virus became the primary control. In 1968, cabbage insects and control practices were evaluated at several commercial vegetable farms in St. Louis County. Most growers were routinely making eight or more applications of synthetic chemical insecticides to their spring crop. We concentrated our efforts at one farm, hoping that management techniques developed at this farm would be disseminated by observation and word of mouth to the other growers in the area. Based on weekly monitoring of the spring cabbage, chemical pesticides were needed only twice, a 75% reduction over previous years. In 1969, only one application of *B. thuringiensis* was required. In 1970, based on observations made every other week at two truck farms, one grower harvested 50% of his cabbage crop without any treatments and the remainder of his crop required one application of *B. thuringiensis*. Another grower used one application of *B. thuringiensis* on the spring crop, and additional applications were made to the summer cabbage sprouts and fall cabbage crop at about three-week intervals. Thus, only six applications of *B. thuringiensis* were used for the seven-month growing season, versus approximately 20 applications of chemical insecticides in previous years. The cost per hectare for materials and labor for either chemical insecticides or *B. thuringiensis* was about $30. Thus, elimination of 14 applications provided a savings of $420 per hectare. We concluded that one or two timely applications of *B. thuringiensis* could protect the spring cabbage and probably not more than six or seven applications would be needed for the seven month growing season. This schedule represents a significant reduction in insecticide use and had no deleterious effects on cabbage production.

Ideally, *B. thuringiensis* should be used to keep populations of pests below given economically damaging levels and should avoid killing 100% of the pests. This allows for the continuous additive suppressive effects of the background beneficial populations to operate, thus reducing the number of applications of *B. thuringiensis* needed. During the development phase of the BC-IBM system, decisions on when to apply *B. thuringiensis* were based primarily on field experience and biological logic rather than on fixed threshold levels. During the 1971 season we conducted field studies to determine economic injury levels and established the following treatment levels: for transplants; treat when large (>7mm) larvae reach 0.3 per plant and when medium (3–7mm) and small (<3mm) larvae reach 1.0 per plant. After the eight-leaf stage, for the head area, treat when any large larvae are observed, and when 0.1 medium or small larvae are found; on the outer leaves treat when large larvae reach 1.0, medium 3.0, and small 6.0 per plant. Counts should be based on the examination of 20 plants per field. From 1970 to 1979 new chemical insecticides were available and most growers used them on a regular schedule. However, two growers on the *B. thuringiensis* system made approximately 1/3 the number of applications. In 1977 these two were the

only growers that were able to control CL; insecticide resistance was developing and was a serious problem by 1979 (Wilkinson *et al.*, 1983). This provided an opportunity to implement the system that had been developed 10 years earlier.

***B. thuringiensis* program implementation**

We initiated a program to implement the *B. thuringiensis*-IPM system in 1980. A three year plan was developed based on the judicious use of *B. thuringiensis* coupled with a scouting program. Year one focused on grower education with active participation by a few growers and the full cost of the scouting program was covered. In year two, the number of growers and acreage would increase and the growers would share the scouting costs. The primary goal of the year three, besides fine-tuning the program was to have the growers pay the full cost of scouting. In 1980 six growers participated with 25 ha of spring cabbage and 12 ha of fall cabbage. Each grower had 1–10 ha within two to six fields at each farm location. Insect populations were monitored weekly by a field scout; whole-plant examinations were made on 20 plants per field regardless of field size by walking a diagonal X transect and selecting plants at approximately uniform intervals. Applications were often restricted to certain fields or portions of specified fields; blanket treatments were seldom required. For the entire growing season of 1980, growers used about one-half as many applications of *B. thuringiensis* on their cabbage as they had of various chemicals during the 1979 season. During 1981 the program expanded to 54 ha and nine growers and in 1982 they had a total of 59 ha of cabbage, broccoli and cauliflower. Results in 1981 and 1982 were comparable to 1980.

Following the switch to the *B. thuringiensis*-IPM system there was an immediate decrease in the number of treatment applications required, and then during a 3–5 year period we saw a continued decrease in the number of *B. thuringiensis* applications. This occurred because there was an increase in background beneficial activity as the local agroecosystem stabilized. Following the 3-year program on education, demonstration, and implementation of the *B. thuringiensis*-IPM program, a private consultant took over the program.

Augmentation program – development

The next step to improving this management scheme required developing technology and procedures for adding beneficial agents through seasonal inoculations early in the crop season when pest populations were low. In 1986 we initiated this third phase; maximizing the use of biological control agents with focus on parasitoids. This phase was conducted at Yakima, Washington. Prior to initiation of this phase we carried out basic population studies to establish the database needed before initiating the field evaluation of beneficial species that would be part of the augmentation program. We focused on establishing this baseline information at several locations in Washington and Oregon (Biever 1992, Biever *et al.*, 1992) Existing laboratory facilities were modified and developed to accommodate a rearing area for production of the three host species and nine parasitoid species. Initial stocks of all but one parasitoid species were collected locally; *Cotesia plutellae* Kurdjumov was introduced from Hawaii. The three lepidopteran pest species, ICW, DBM, and CL, were reared using the same laboratory diet (Berger, 1963) for both colony production and to provide host material for parasitoids. Colonies of the following parasitoid species were established: *Diadegma insulare* (Cresson), *Oomyzus sokolowskii* (Kurdjumov), *C. plutellae, Microplitis plutellae Musebeck, Pteromalus puparum* (L.), *Phryxe vulgaris* (Fallen), *C. rebecula Marshall, Voria ruralis* (Fallen), and *C. marginiventris* (Cresson). In 1986 and 1987 we established colonies and developed rearing, production, and handling procedures for three pest and nine parasitoid species. From 1988 through 1990 we concentrated on evaluating and integrating a number of parasitoid species to regulate the pest complex with emphasis on population monitoring and early season inoculative releases of the parasitoids. In 1988 we conducted the first field releases of pests and parasitoids in isolated plots of cabbage; each plot consisted of 1,000 plants. The treatments were: pests only and two release rates of parasitoids (25 and 50 pairs per species per plot). We also developed handling, storing, and release techniques. In 1989 tests were conducted in isolated blocks of broccoli with seven species of parasitoids and we evaluated four treatments: pests only and parasitoids at three rates (150, 300, and 600 pairs/species/plot). Pest (adults) were released on all plots on 2 dates (17 days apart) at a rate of 250 pairs per species. Plots were separated by at least 1.6 k and consisted of 0.4 ha of broccoli. All three parasite rates reduced populations of the pest species. At harvest, plots with the two highest release rates had the least insect damage (less than 5%); and the control had the most damage (approximately 25%). Essentially, all broccoli heads from the release plots were marketable (U.S. Grade No. 1) because our ratings were conservative and considered the slightest damage that might have been caused by an insect. In 1990 we evaluated two treatments: pests only and pests plus two releases of nine species of parasitoids (70–400 pairs/0.4 ha/species). Plots with parasitoid releases had higher rates of parasitization (>80% by midseason) and reduced pest populations. We concluded that early season inoculations with parasitoids could reduce the number of applications of *B. thuringiensis* required and in some cases eliminate the need for *B. thuringiensis* applications.

Augmentation program-implementation

In 1990, a series of fortunate circumstances provided the opportunity for our BC-IPM program to move to commercial grower's fields. Buddy Madgen, owner and operator of BioFac, a commercial insectary in Mathis, Texas, was interested in developing a

parasitoid release program for cabbage in the Rio Grande Valley of Texas as he saw an impending need for an alternative management approach. Growers had reached a crisis situation and could no longer effectively suppress populations of the DBM because of insecticide resistance. During the previous 6 years, cabbage production had declined significantly in the Rio Grande Valley primarily because of the inability to control DBM.

BioFac began parasite production and contracted with cabbage growers to participate in the biocontrol release program during the November 1990–April 1991 growing season. To support the program we conducted field-population monitoring of insect populations, information and assistance on rearing and release of beneficials, and documented the program under commercial field conditions.

The program BioFac implemented was a modification of the BC-IPM system we had developed, and was only concerned with two lepidopteran species, DBM and the CL. They released *C. plutellae*, and *D. insulare* for DBM, *C. marginiventris* and *Trichogramma pretiosum* Riley for CL, and the predator *Chrysoperla rufilabris* (Burmeister) for the lepidopterans, whiteflies, and aphids. DBM was expected to be the dominant pest; however, it turned out that the CL was the primary pest. This may have been a fortunate occurrence as it demonstrated to the growers and to BioFac the importance of a monitoring program to establish appropriate control tactics, particularly when these tactics are species specific. BioFac's program utilized regular releases of the beneficials, rather than tailoring them to scouting based decisions; thus, more beneficials were released than needed and, at times, releases were made when suitable host stages were not present. When implementing a new type of management strategy such as this, it is probably better to release too many rather than too few beneficials. Growers also are used to having insect management procedures applied on a regular basis; thus, scheduled releases provide them with a sense of security. Our premise was that if a scheduled release system could be cost effective and accepted by the growers, then the next step would be to release only when needed and, thus, reduce costs.

The program involved five growers and 53 ha of cabbage (eight fields) that were monitored weekly. Treatments were: release of beneficial agents only, release of beneficial agents plus *B. thuringiensis* when needed, and release of beneficial agents plus *B. thuringiensis* and chemical insecticides (fields put into the program after receiving one or more applications of chemical insecticides). Seventy-five percent of the fields that received releases of beneficial agents also received one application of *B. thuringiensis*. Cabbage fields not in the release program, but in the same geographical area, received between 5 and 26 applications of chemical insecticides. The cost of using the beneficials compared favorably with insecticide treated fields and provided satisfactory crop protection. Insect damage at harvest was evaluated at two grower locations. The average damage for fields treated with chemical insecticides was 1.4 % and for the BC-IPM fields it was 0.6 %. The average cost for the chemical insecticide treated fields was $390/ha and for the BC-IPM it was $395/ha. Although far from perfect, the field project demonstrated that a BC-IPM system that emphasizes augmentative releases of a number of beneficial species can regulate a complex of pest species.

A parasite release program utilizing *C. plutellae* for control of DBM has been in operation in Guatemala for three years and for two years in Mexico. Three to six releases of 500-1000 pairs/ha were made; rates depended on population pressures. BioFac initiated the program in Guatemala in October 1993 and through March 1994 more than 300 ha of broccoli and cauliflower were protected by this system. During the 1994–95 season the program included about 600 ha in Mexico and 250 ha in Guatemala. Releases were limited in 1996 as logistics of transporting the parasitoids from the USA to the other countries has become difficult and growers are often relying on routine applications of *B. thuringiensis*, thus we are already observing the reduced effectiveness of this biological insecticide in Mexico where many generations of DBM have been treated with *B. thuringiensis* during the last 9 years. In both Guatemala and Mexico a complex (different for each country) of lepidopteran pests occur at various times throughout the production season and these were effectively suppressed with timely applications of *B. thuringiensis* plus the native parasitoid populations. DBM is the primary pest of crucifers in Mauritius and is particularly damaging to cauliflower and cabbage. Insecticide resistance was first noted in the early 1980's and required the use of new replacement insecticides. In 1991 an integrated pest management program for DBM was initiated (S. I. Seewooruthun MOA, Reduit, Mauritius, unpublished data). Two parasitoid species, *C. plutellae* and *Diadegma semiclausum* Hellen, were introduced from the Asian Vegetable Research and Development Center, Taiwan in October 1991. Since the initial introduction, *C. plutellae* adults (over 9000) have been reared from field collected DBM larvae and released throughout the growing areas in Mauritius. *C. plutellae* is well established (parasitism is often 70–90%) in all crucifer production areas while *D. semiclausum* is rarely found (C. Dunhawoor, MOA, Reduit, Mauritius, unpublished data).

More recently a project has been initiated to utilize augmentative releases of parasitoids to suppress DBM populations as part of an overall program involving the release of sterile DBM. This program is being supported by IAEA, Vienna, Austria. During 1996 baseline population data is being collected from all production areas on pest and beneficial insects; a rearing facility for the production of DBM and parasitoids has been built, personnel have been trained for rearing on artificial diet and before the end of the year a series of demonstration/small field studies will be conducted. These fields will receive inoculative

releases of *C. plutellae* and the judicious use of *B. thuringiensis* if needed. This product has not been used in Mauritius, thus if we initiate a program of grower use which is one of only making applications of *B. thuringiensis* when needed and as part of a BC-IPM system the resistance to *B. thuringiensis* should be avoided. Later phases of the program will include evaluation of the combination of inherited sterility and the inundative release of *C. plutellae*.

In developing specialized BC-IPM management programs it is important to remember that the systems are dynamic and under constant change and evolution, thus regular monitoring is essential. However, to be effective, grower education and acceptance are necessary along with the field monitoring and timely recommendations on control measures.

References

Berger, R. S. (1963). Laboratory techniques for rearing *Heliothis* species on artificial medium. USDA-ARS (Report) 33–84.

Biever, K. D. (1992). Distribution and occurrence of *Cotesia rubecula* (Hymenoptera:Braconidae), a parasite of *Artogeia rapae* in Washington and Oregon. *J. Econ. Entomol. 85:* 739–742.

Biever, K. D., R. L. Chauvin, G. L. Reed and R. C. Wilson (1992). Seasonal occurrence and abundance of lepidoptera pests and associated parasitoids on collards in the northwestern United States. *J. Entomol. Sci. 27:* 5–18.

Biever, K. D., D. L. Hostetter and J. R. Kern (1994). Evolution and implementation of a biological control-IPM system for crucifers: 24-year case history. *Amer. Entomol. 40:* 103–108.

Wilkinson, J. D., K. D. Biever, and D. L. Hostetter (1983). Resistance of the Cabbage Looper, *Trichoplusia ni* (Hubner) (Lepidoptera: Noctuidae) to Methomyl in Missouri. *J. Kans. Entomol. Soc. 56:* 59–61.

A *Plutella/Crocidolomia* management program for cabbage in Indonesia

B. M. Shepard[1] and N. A. Schellhorn[2]

[1]*Clemson University, Coastal Research and Education Center, 2865 Savannah Highway, Charleston, South Carolina, U.S.A., Presently Team Leader - Clemson Palawija IPM Project, Jl. Heulang, No. 11, Bogor, 16161, Indonesia*

[2]*Department of Entomology, University of Minnesota, Saint Paul, Minnesota, 55108-6125 U.S.A.*

Abstract

The importance of the parasitoid, *Diadegma semiclausum* Hellen, in suppressing populations of the diamondback moth (DBM), *Plutella xylostella* Linn., is generally accepted. However, when DBM is under control by the parasitoid, i.e., when chemical insecticides are reduced or eliminated, the cabbagehead caterpillar (CHC), *Crocidolomia binotalis* Zeller, often increases, thus causing farmers to apply chemical insecticides. This, in turn, causes DBM to resurge due to destruction of *D. semiclausum* and other natural enemies. Intensive surveys in major crucifer growing areas in Indonesia have revealed few CHC parasitoids or entomopathogens. Likewise, indigenous predators are not able to maintain populations of CHC below seriously damaging levels.

The small cabbage fields (< 0.5 ha) in Indonesia allowed us to determine if hand-picking leaves with CHC egg masses and larval clusters, plus spot spraying with *Bacillus thuringiensis* Berliner (B.t.), is a practical approach for managing both caterpillar species. Using these techniques only 7 or 8 spots sprays of B.t. were made compared to as many as 26 chemical sprays, the usual farmer practice. Thus, hand-picking leaves with CHC eggs and larvae plus spot spraying B.t. should stabilize the system by conserving natural enemies of both DBM and CHC and may be a practical solution to managing the caterpillar complex in highland crucifers in Indonesia or other countries with small farm holdings.

Key words: *Plutella, Crocidolomia,* cultural control, parasitoids, B.t.

Introduction

There are about 67,000 hectares planted to cabbage in Indonesia with a total production of nearly 1.5 million tons (Biro Pusat Statistik, 1994). A number of diseases can be locally important but the major production constraints are the diamondback moth (DBM), *Plutella xylostella* and the cabbagehead caterpillar (CHC), *Crocidolomia binotalis*. *Hellula* sp. can be important in lowland cabbage and *Helicoverpa armigera* (Hubner) is sometimes a serious pest in highland areas of Java and Sulawesi. The introduction and establishment of the parasitoid *Diadegema semiclausum*, against DBM in Indonesia is well documented (Vos, 1953, Sastrosiswojo and Eveleens, 1977; Sastrosiswojo and Sastrodihardjo, 1986). DBM is usually kept in check by this parasitoid when chemical insecticides are not used but farmers who have not undergone IPM training still treat their cabbage fields on a calendar basis. This, in turn, causes DBM to resurge but usually keeps CHC under control. Thus, it is essential to consider CHC and DBM together in a management program (Sastrosiswojo and Setiawati, 1992; Waterhouse, 1987).

Unlike DBM, natural enemies of CHC are not able to keep this pest in check in Indonesia. Earlier surveys of CHC by Othman (1982) in Cipanas, West Java, revealed the parasitoids *Diadegma* (=*Inareolata*) sp. and *Sturmia* (=*Bleparipa*) sp., both parasitizing at levels below 10%. Low numbers of *Inareolata (=Diadegma) argenteopilosa* Cam. (Ichneumonidae) and *Sturmia inconspicuoides* Bar. (Tachinadae) were reported by Sastrosiswojo and Setiawati (1992). Likewise, low species diversity and numbers of parasitoids were found in collections in several areas of Indonesia (Annual Report, 1995). Information on the action of predators of CHC is limited to a few anecdotal sightings.

Nomuraea rileyi (Farlow) Samson is the major entomopathogen attacking CHC in Indonesia but epizootics usually occur after the crop has sustained severe damage (Annual Report, 1995). Occasionally microsporidian infected larvae are collected from the field (Carner and Suryawan, 1993).

Suggested methods for CHC control include various trap crops (Prabaningrum and Sastrosiswojo, 1994, Srinivasan and Krishna Moorthy, 1991) or intercropping with crops such as tomato (Sastrosiswojo and Setiawati, 1992). Neem (*Azadirachta indica* A. Juss.) has been shown to have activity against CHC (Fagoonee and Lauge, 1981) but all of the above methods usually are not practical in the Indonesian vegetable production system. Clearly, a control strategy for DBM must also include CHC and the importance of conserving natural enemies, especially *D. semiclausum* for DBM control, is critical for a successful IPM program for both pest species.

We surveyed cabbage in all major production areas of Indonesia to assess the level of parasitism and infection by entomopathogens on DBM and CHC. Then we carried out field studies to determine if hand-picking leaves with egg masses and larval clusters along with spot spraying *Bacillus thuringiensis* (*B.t.*) could be a practical approach for DBM and CHC management.

Methods and Materials

Field surveys/collections: Baseline levels of parasitism and infection by diseases were determined by collecting over 1,200 of each species from cabbage fields in Java, Sumatra, Sulawesi and Bali. Larvae were held in small plastic cups with artificial diet and monitored for parasitism and disease incidence from November, 1994 and May 1996. In addition, more than 150 egg masses were collected in cabbage fields near Barastagi, North Sumatra to determine parasitism.

Hand-picking eggs/larval clusters: Two field tests were carried out to determine if hand-removal of leaves with CHC egg masses and larval clusters plus spot treating with *B.t.* (Dipel®) could be a viable control tactic. Both sites were located in highland vegetable areas with both DBM and CHC targeted by farmers in the areas as major pests. Fields of cabbage (*Brassica oleraceae* L., var. 'capitata') were examined twice per week (at 2–3 day intervals) in both tests. The first test was conducted in Alahan Panjang, West Sumatra from April through July, 1995. Treatments included: 1.) hand-picking CHC egg masses and larval clusters up to 30 days after transplanting seedlings, then hand-picking plus spot spraying with *B.t.* after CHC larvae had migrated from the larval cluster, 2.) hand picking throughout the season and spraying the entire plot with B.t.; 3.) standard farmer practice (weekly applications of chemical insecticides), and; 4.) untreated control. A small hand-held mist sprayer was used to apply the *B.t.*.

The second test was conducted in Kuta Tengah near Barastagi, North Sumatra from May through July, 1995. Four week old cabbage were planted 40 cm apart on 25 April, 1995. Treatments for the second test included: 1.) the usual farmers' practice in the area, i.e., application of chemical insecticides, usually Decis® and Padan®, twice weekly, 2.) hand picking leaves with egg masses and larval clusters of CHC up to 42 days and spot spraying with B.t. thereafter (unless there was more than 0.5 DBM or more than two CHC egg masses determined from a 20 plant sample taken by sampling a "U" shaped pattern across the plot). If numbers exceeded these limits, the entire plot was sprayed with *B.t.,* 3.) applying *B.t.* to the entire plot if DBM and CHC populations were as in treatment 2, and, 4.) untreated control. Yields were determined by taking 20 plants, sampled in a "U" pattern across the field. A backpack sprayer was used to apply the *B.t.*. At harvest, 20 plants were taken from each plot and the quality was rated as: 1.) export (1 kg or more), 2.) for sale to local markets (less than 1 kg) or, 3.) unmarketable.

Plot sizes for both tests were 10 X 10 m^2 with 3 replicates. Egg masses and larval clusters were located by examining plants while walking slowly between two cabbage rows. Eggs and larvae were removed by picking the leaf that contained them. Time required to sample each plot was recorded for 25 sampling dates (May 5–July 28, 1995).

Data were transformed using log normal transformation to meet the assumptions of homogeneity of variance and normality and subjected to analysis of variance (ANOVA). Means were separated using Tukey's test at $P < 0.05$ (SAS Institute 1989).

Results

Survey Results: Approximately 1200 DBM larvae were collected and held for observation, during November 1995 and October, 1996. Of these, parasitism levels by *D. semiclausum* averaged about 75%, with maximum parasitism reaching over 90%. On two occasions in North Sulawesi (Kecamatan: Modoinding), there were epizootics of the entomopathogen, *Zoophthora radicans* (Brefeld). Most of the larvae were already dead at the time surveys in the area were being conducted, thus low numbers of live larvae were collected. Of these (n=61) over 95% died from the disease. Clearly DBM was under good control by natural enemies in these fields. Our general observations were that dense DBM populations were always indicative of frequent insecticide use by farmers and when CHC numbers were high, fields were sprayed less frequently or not at all.

CHC larval collections (n=1200) indicated that indigenous parasitoids and entomopathogens were not maintaining its populations below satisfactory levels. Egg parasitoids (probably *Trichogramma* spp.) was found near Barastagi, N. Sumatra, but only rarely. *Pristomerus* sp. (Hymenoptera: Ichneumonidae) attacked up to about 20% of CHC larvae at Malino, S. Sulawesi, but only on one occasion. Low numbers of *Eriborus sinicus* (Holmgren) (Hymenoptera: Ichneumonidae), *Bleparipa* (=*Sturmia*) and *Argyropylax* sp. (Diptera: Tachinidae) were routinely found in CHC collections from other locations in Indonesia but the level of parasitism by all species was almost always below 10%. We routinely encountered leaves with feeding damage but no CHC larvae present. It is likely that predators are a major source of mortality if allowed to operate without chemical insecticides. The incidence of infection by entomopathogens was, in general, low. However, high levels of infection by *N. rileyi* occurred on occasion but only when larval populations were dense and the crop had sustained considerable damage.

Hand-picking CHC eggs/larval clusters: Egg masses were almost always deposited on undersides of lower leaves and were sometimes difficult to locate, especially in older plants. However, after eggs hatch (7–8 days) larvae feed as a cluster in the same area on the same leaf until about the third instar (usually about 4 days after hatching). The feeding damage by these larval clusters was distinct and easily recognized as the sampler walked slowly through the field between rows. Average time to sample each 10 X 10 m^2 plot was about 15 minutes.

In Alahan Panjang, using hand picking and spot treatments, over 90% of the cabbage heads were rated as marketable. The farmers' usual practice yielded results similar to hand picking and spot spraying but 8 chemical sprays were applied in the farmers® practice treatment compared to only 7 spot treatments with *B.t.* We concluded that the small hand-held sprayer did not provide adequate plant coverage. Results may have been better if *B.t.* had been applied using a backpack sprayer. In the untreated control, nearly 40% of the heads were unmarketable.

There were three distinct peaks (June 13, July 4 and July 14) in the CHC egg mass/larval populations in Kuta Tengah *(Figure 1)*. CHC egg mass and larval population levels were significantly higher in the untreated controls than in any other treatments. Other treatments were not significantly different. Likewise,

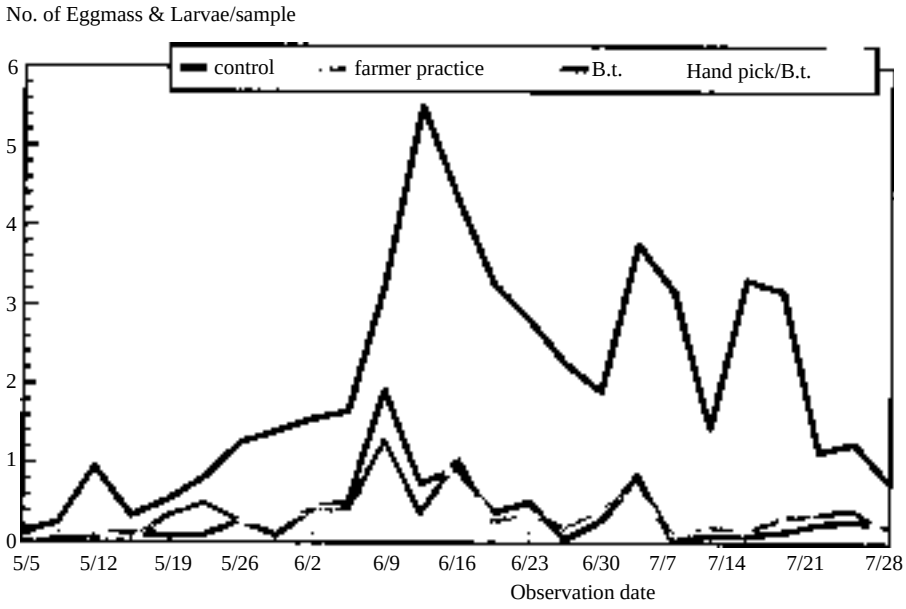


Figure 1. Seasonal fluctuation of CHC eggs and larval clusters (number per 20 plant sample) on cabbage in Kuta Tengah, North, Sumatra, Indonesia. 1995.

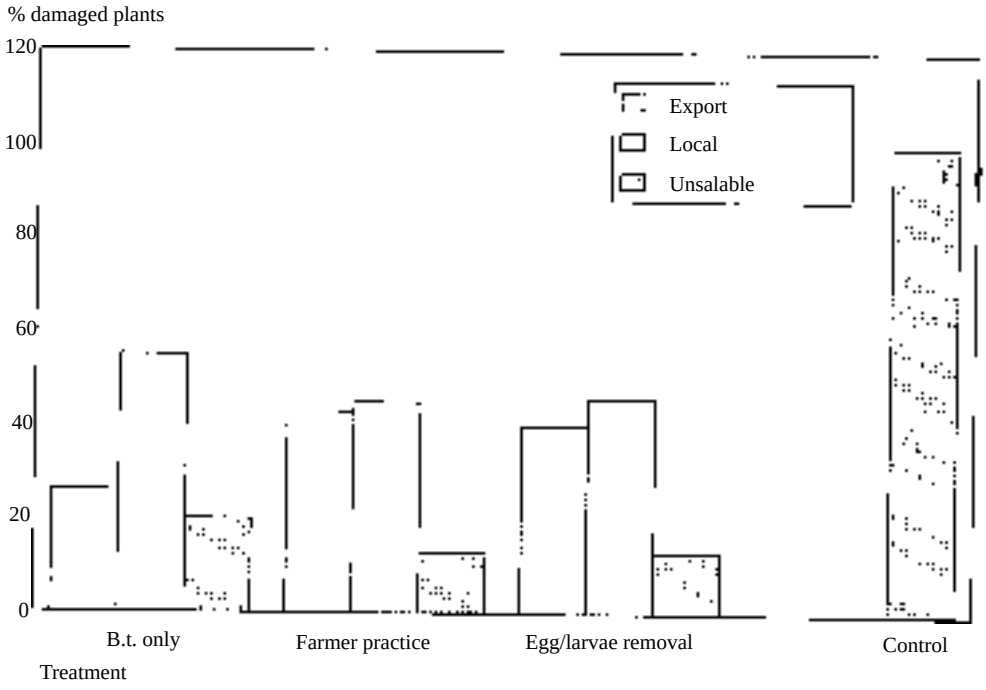


Figure 2. Percent of cabbage plants sold to local markets (< 1 kg), for export markets (>1 kg) or unmarketable due to damaged by CHC. Kuta Tengah, North Sumatra, Indonesia. 1995.

except for untreated control plots, there was no difference in numbers of damaged plants from samples taken in the three plots *(Figure 2)*. The percent of unmarketable plants was about the same in all treatments except for the untreated control plots where 100 per cent of the plants were considered unmarketable. There were fewer cabbages for export in the *B.t.* treated plots but this difference was not significant (P>0.05) according to Tukey's test. The most significant aspect of the treatments is that the usual farmers' practice treatment required 26 applications of chemical insecticides. *B.t.* sprays were applied 8 times in treatment 3 (hand picking CHC plus treating the entire plot with *B.t.* if more than 0.5 DBM or two egg masses or larval cluster of CHC were found in a 20 plant sample). However, only 7 spot sprays with *B.t.* were required in the hand picking treatment. DBM populations were low in all plots throughout the study with populations averaging well below 1 larva per plant. Thus, it is likely that all sprays for DBM could have been avoided without significant crop loss.

The profitability of hand picking eggs and larval clusters plus spot spraying with *B.t.* may be best utilized in areas where cabbage fields are small and not much time is required to find and remove eggs and larval clusters. In addition to conserving natural enemies that help control both DBM and CHC, resistance to *B.t.* could be slowed considerably using only spot sprays.

Discussion

Results from surveys of DBM and CHC indicated that the former is under good biological control where chemical sprays can be avoided. Communities of natural enemies of CHC, however, are at low densities and are not able to keep this pest under natural control. Results from the two field tests indicated that hand-picking leaves with CHC egg masses and larval clusters plus spot spraying with *B.t.* may be viable approach for controlling CHC in cabbage while conserving communities of natural enemies, especially for *D. semiclausum* which help keep DBM under control when chemical insecticides are not used. Clearly, the approach is more suitable for small farm holdings (like those in Indonesia) where time required to search for and remove eggs and larval clusters is minimal.

The threshold concept is not appropriate using this approach. Rather, continuous surveillance is required at close intervals (approximately every 3 days). Even so, time and money saved using the hand-picking/*B.t.* spot spray method should make it appealing to farmers, especially those who have undergone IPM training and appreciate the value of natural enemy conservation. Hand picking CHC egg masses and larval clusters plus spot spraying *B.t.* eliminates attendant problems associated with using chemical insecticides.

Acknowledgments

Information reported herein resulted from a combined effort of the Clemson University Palawija IPM team consisting of the senior author, Dr. E. F. Shepard, Dr. M. D. Hammig, Dr. G. R. Carner, Dr. S. G. Turnipseed (Clemson University), Ir. Samsudin and Widji Soekirno (Bogor Institute for Food Crops Biotechnology). We are grateful to our collaborators, both formal and informal, for their assistance, especially with conducting field studies. These include: Dr. Russ Dilts (FAO), Dr. I. N. Oka (FAO and Ministry of Agriculture advisor), Dr. Aunu Rauf (Institut Pertanian Bogor), Dr. Ann Braun (International Potato Center), Ir. Djoni and Ir. Zamzami (BPTPH laboratory in Padang, West Sumatra), Mr. Paul Musante and Ir. Budiman Saragih (World Education). We appreciate the assistance of Mr. Alberto Barrion, Division of Entomology and Plant Pathology, the International Rice Research Institute and Dr. Steven Heydon, Department of Entomology, University of California, Davis, California, for their assistance with parasitoid identifications. We are especially thankful to our sponsor, the United States Agency for International Development and Dr. Andrea Yates and Mr. Prijanto Santoso (U.S.A.I.D.) for their assistance in many ways and for support of our work. The Henry Luce Foundation provided financial support for N. A. Schellhorn. We extend our sincere thanks for this support. Finally, but most importantly, we thank our farmer cooperators, especially Pak Agustoni.

References

Annual Report (1995). Integrated Pest Management Research, Development and Training Activities for Palawija Crops in Indonesia. U.S.A.I.D. Project No. 497–057. Clemson University. 102 pp.

Biro Pusat Statistik (1994). Agricultural Survey Production of Secondary Food Crops in Indonesia. Jakarta, Indonesia. 223 pp.

Carner, G. R. and I. B. G. Suryawan (1993). Viral and protozoan pathogens of insect pests of palawija and vegetable crops. In: Prosiding Makalah, Simposium Pathologi Serangga I, Yogyakarta, Indonesia. pp. 54–60.

Fagoonee, I. and G. Lauge (1981). Noxious effects of neem extracts on *Crocidolomia binotalis. Phytoparasitica. 9(2):* 111–118.

Othman, N. (1982). Biology of *Crocidolomia binotalis* Zell. (Lepidoptera: Pyralidae) and its parasites from Cipanas area (West Java). SEAMEO Regional Center for Tropical Biology. Bogor, Indonesia. 52 pp.

Prabaningrum, L. and S. Sastrosiswojo (1994). The use of Indian mustard and rape as trap crops for *Plutella xylostella* and *Crocidolomia binotalis* on cabbage. Internal Report. Lembang, Horticultural Research Institute. 8 pp.

SAS Institute (1989). SAS/STAT users' guide, version 6. Fourth edition. SAS Institute, Cary, North Carolina, USA.

Sastrosiswojo, S. and K. G. Eveleens (1977). Biological control of *Plutella xylostella* on cabbage in Indonesia by the introduced parasite *Diadegma eucerophaga. Bull. Penel. Hort. V (6):* 23–31.

Sastrosiswojo, S. and S. Sastrohidhardjo (1986). Status of biological control of diamondback moth by introduction of parasitoid *Diadegma eucerophaga* in Indonesia. *In:* Talekar, N. S. and Griggs, T. D. (eds.). Diamondback Moth Management. Proceedings of the First International Workshop, Asian Vegetable Research and Development Center, Tainan, Taiwan. 185–194.

Sastrosiswojo, S. and W. Setiawati (1992). Biology and control of *Crocidolomia binotalis* in Indonesia. *In:* N. S. Talekar (ed.). Diamondback Moth and Other Crucifer Pests, Proceedings of the Second International Workshop. Asian Vegetable Research and Development Center, Tainan, Taiwan, December, 1990. 81–87.

Srinivasan, K. and P.N. Krishna Moorthy (1991). Indian mustard as a trap crop for management of major lepidopterous pests of cabbage. *Tropical Pest Management. 37 (1):* 26–32.

Vos, H. C. C. A. A. (1953). Introduction in Indonesia of *Angitia cerophaga* Grav., a parasite of *Plutella maculipennis* Curt. Pemberitaan Balai Besar Penjelidikan Pertanian, Bogor, Indonesia. 31 pp.

Waterhouse, D.F., and K. R. Norris (1987). Biological Control: Pacific Prospects. Inkata Press, Melbourne, 454 pp.

Technology transfer of *Cotesia*-based IPM for diamondback moth on lowland crucifers in the Philippines

B. Morallo-Rejesus[1], E. L. Inocencio[2], J. E. Eusebio[3], and S. F. Barroga[4]

[1 & 2]Department of Entomology, University of the Philippines at Los Banos, College, Laguna, Philippines and [3]Crop Research Division, PCARRD, Los Banos, Laguna, Philippines

[4]Mariano Marcos State University, Batac, Ilocos Norte, Philippines

Abstract

The components of the integrated pest management (IPM) technology for diamondback moth (DBM), *Plutella xylostella* in the lowland are the release of larval parasitoid, *Cotesia plutellae* supplemented with selective insecticide through training of trainers (technicians of the local government units (LGU) and researchers of state colleges and universities (SCU) and farmers followed by farmer participated demo farms (FPDF). The training was conducted in cooperation with LGU, SCU and farmer associations.

A total of 1 154 farmers and 176 trainers from 31 municipalities of 9 provinces of Batangas, Camarlines Sur, Cavite, Ilocos Sur, Ilocos Norte, Laguna, Nueva Ecija, Rizal and Quezon were trained and 49 FPDF were set-up in 7 provinces.

High *C. plutellae* parasitism and cocoon formation and lower DBM population were noted in IPM than FCP fields. The farmer adoption of IPM technology resulted in the increase net income ranging from US$2 781.25 to US$10 984.46/ha, 5–78% higher than FCP due to lower production cost and higher yield. The production cost per hectare range from US$1 104.16–3 902.34, 2–45% lower than the FCP. Insecticide application was reduced from 1–4 times in Quezon and to 3–9 in Ilocos Sur and Norte from 15–36 times before the piloting of the technology. The increase net income varied with the location, season, production cost and price of cabbage.

Seventy to 100% of the cooperators regularly visited by the AVNET personnel and trainers implemented properly the IPM technology. While cooperators that were not regularly visited only 30% succeeded.

Key words: *Cotesia plutellae, Plutella xylostella,* IPM, parasitoids, technology.

Introduction

In the Philippines, the diamondback moth (DBM), *Plutella xylostella* (Linn.) was first observed as the most destructive pest of crucifers in the highland in 1960 (Barroga, 1967) and in the lowland in 1970 (Cadapan and Gabriel, 1972). Since then, farmers relied heavily on chemical application to control this insect pest leading to the development of resistance to organophosphates, carbamates and pyrethroids (Barroga and Morallo-Rejesus, 1975; Cardona, 1986, Ebuenga, 1992).

This leads farmers to spray frequently (15–36 times) during the cropping season with mixed insecticides at doubled the recommended rates. These practices aggravated the resistance and residue problems in the Philippines. Same problems were reported in Asia, thus, the search for biological control agents for the control of DBM ensued. The introduction of natural enemies of DBM in Malaysia (Ooi and Lim, 1989); Indonesia (Sastorosiswojo and Sastrodihardjo, 1986) and Taiwan (Talekar, 1990; Talekar *et al.* 1992) resulted in significant decline in DBM population. Lim (1986) reported that although the solitary larval parasitoids, *Diadegma semiclausum* (Hellen) and *Cotesia (Apanteles) plutellae* (Kurdj.) have the greatest potential in the control of DBM, but their effect is not enough if used alone. Their most important use is as a component of an IPM program or in combination with other natural enemies like an effective pupal or egg parasitoid of DBM.

The integration of the use of microbial insecticides and the parasitoids, *D. semiclausum* and *C. plutellae*, was reported as promising in the highland and lowland areas of Taiwan by Talekar (AVRDC, 1990). Subsequently, these parasitoids were introduced into the Philippines in 1989 from Taiwan through the Asian Vegetable Network (AVNET Phase I) collaborative project coordinated by the Asian Vegetable Research and Development Center (AVRDC), Taiwan with the following participating countries: Thailand, Indonesia, Malaysia, and the Philippines. Locally, the project was coordinated by the Philippine Council for Agriculture Forestry and Natural Resources Research and Development (PCARRD).

In the AVNET Phase I, the viability of the *Cotesia*-based IPM technology to control DBM was evaluated and demonstrated in farmers' field in lowland elevation (400–800 meters above sea level) of Laguna, Batangas, Quezon and Cavite from October 1989 to April 1993 (Morallo-Rejesus *et al.*, 1994). The technology reduced DBM population and insecticidal applications, and increased the net income of farmers.

In May 1993, the project on IPM of Cruciferous Insect Pests (AVNET Phase II) was implemented which aimed to further pilot and promote the concept

and technology of *Cotesia*-based IPM for the management of DBM in other lowland areas of Luzon, Philippines.

The Cotesia-based IPM technology for DBM

For the lowland elevation crucifers, the IPM technology of AVRDC (1990) was modified by incorporating the economic threshold level (ETL), the tool used for deciding when to apply supplemental insecticidal application (Morallo-Rejesus *et al.*, 1994).

The component of the IPM technology are as follows:

A. The release of *C. plutellae* (cocoons/adults) at 3 000–10 000/ha/release ($^1/_2$m^2 or $^1/_6$ plants to $^1/_{12}$ plants) with 3 to 7 releases for the first to second cropping. The number of releases was reduced in the next cropping seasons depending upon the level of parasitism. The rate of parasitism was monitored by collecting 50 or 100 3rd or 4th instar DBM larvae and reared until adult emergence. If the increase of parasitoid population is slow or parasitism is below 75% the same number of parasitoids are released immediately.

B. Spray supplement based on ETL
The ETL are two larvae at seedling to vegetative stage (1–4 weeks after transplanting (WAT) and 5 larvae at vegetative to heading stage (5–12 WAT). It is determined by monitoring the number of DBM larvae 3–4 days on 50–100 plants, X pattern. If ETL is reached, selective insecticides like *Bacillus thuringiensis kurstaki* and B.t. *aizawai* (microbial), fenvalerate (pyrethroid), carbaryl (carbamate), teflubenzuron (IGR) and diafenthiuron (pyrrole) is sprayed.

Strategy for transferring the technology

The *Cotesia*-based IPM technology for DBM was transferred to the farmers by conducting trainers' and farmers' trainings followed by farmer-participated demo farms (FPDF). The trainings were conducted in cooperation with local government unit (LGU), State College and Universities (SCU) and/or farmer associations or cooperatives. In addition, the technology was popularized through the media (radio broadcast, TV, printed materials (bulletins and handouts). A bulletin "Guide to Control Diamondback Moth" in English and Pilipino (Morallo-Rejesus and Sayaboc, 1992) was published and distributed for the first time during the launching of the Farmers' Field School for IPM on DBM in Cordillera Region in February 1992 by the Department of Agriculture (DA) in cooperation with Benguet State University (BSU), La Trinidad and University of the Philippines at Los Banos (UPLB). Papers and posters or exhibits were presented in conferences, workshops and fairs.

Lectures on the IPM technology for DBM were given by the senior author in various trainings/fairs on vegetable production and IPM with total attendance of 865 persons from 31 municipalities.

Fourteen trainers and farmers trainings of one to two days were held in six provinces with 465 participants. In each training site, at least three farmers served as initial cooperation for demonstrating the IPM technology. Each farmer volunteer was requested to set aside 500 to 5 000 m^2 of his crucifer farm with 2–3 weeks old cabbage at the time of training. One half of the field was set aside as the IPM-managed field while the other half as FCP managed field (farmer applied his usual practices). They provided all the inputs except for the *C. plutellae* and selective insecticides for the IPM-managed fields which were provided by the AVNET. In some places, the farmers only provided the IPM fields.

The initial release was done in a field planed with 2–4 weeks old cabbage thereafter at weekly or biweekly interval at the rate of 3 000 to 10 000 cocoons/hectare. In many cases, 3 to 6 releases were made on the first cropping, 2–4 releases on the second cropping and 1 to 3 or no releases in the subsequent cropping depending upon the build-up of the parasitoid population.

Three to four days after each release of the parasitoids, 100 3rd or 4th instar DBM were collected to determine field parasitism. Likewise, the DBM larvae in 50 plants were counted in an "X" pattern; if ETL is reached, the farmer spray with *B.t. kurstaki* (Btk). In the first cropping, the *B.t.k.* or other selective insecticides (teflubenzuron, diafenthiuron) was supplied by the AVNET for the IPM managed field. In the next cropping, the farmer bought their own *B.t.k.*

In Quezon, the AVNET personnel regularly (weekly) visited and assisted the cooperators in the monitoring of DBM and ETL and parasitism determination. In Ilocos Sur, Ilocos Norte, Cavite, Rizal, Nueva Ecija, the AVNET personnel visited the cooperators once in two weeks to release the parasitoids. In these places, LGU technicians and researchers from RCPC or SCU's were relied upon to assist the farmers in the monitoring once or two times a week. All *C. plutellae* population released in the lowland were mass-reared and supplied by the Department of Entomology, UPLB (AVNET Project).

Forty nine farmer participated demo farms were set up in 21 barangays of 14 municipalities in Quezon, Cavite, Ilocos Sur, Ilocos Norte, Nueva Ecija and Camarines Sur *(Figure 1)*. A total of 182 050 *Cotesia* cocoons were released in an aggregate area of 74 850 m^2. Twenty thousand *Cotesia* cocoons were provided to other researchers of government agencies from other localities upon request.

The level of parasitism and establishment of *C. plutellae*

Level of C. plutellae parasitism

The pre-release survey in 1989 indicated the absence of indigenous *C. plutellae* in crucifer-growing areas in mid and lowland elevation (Morallo-Rejesus *et al.*, 1994).

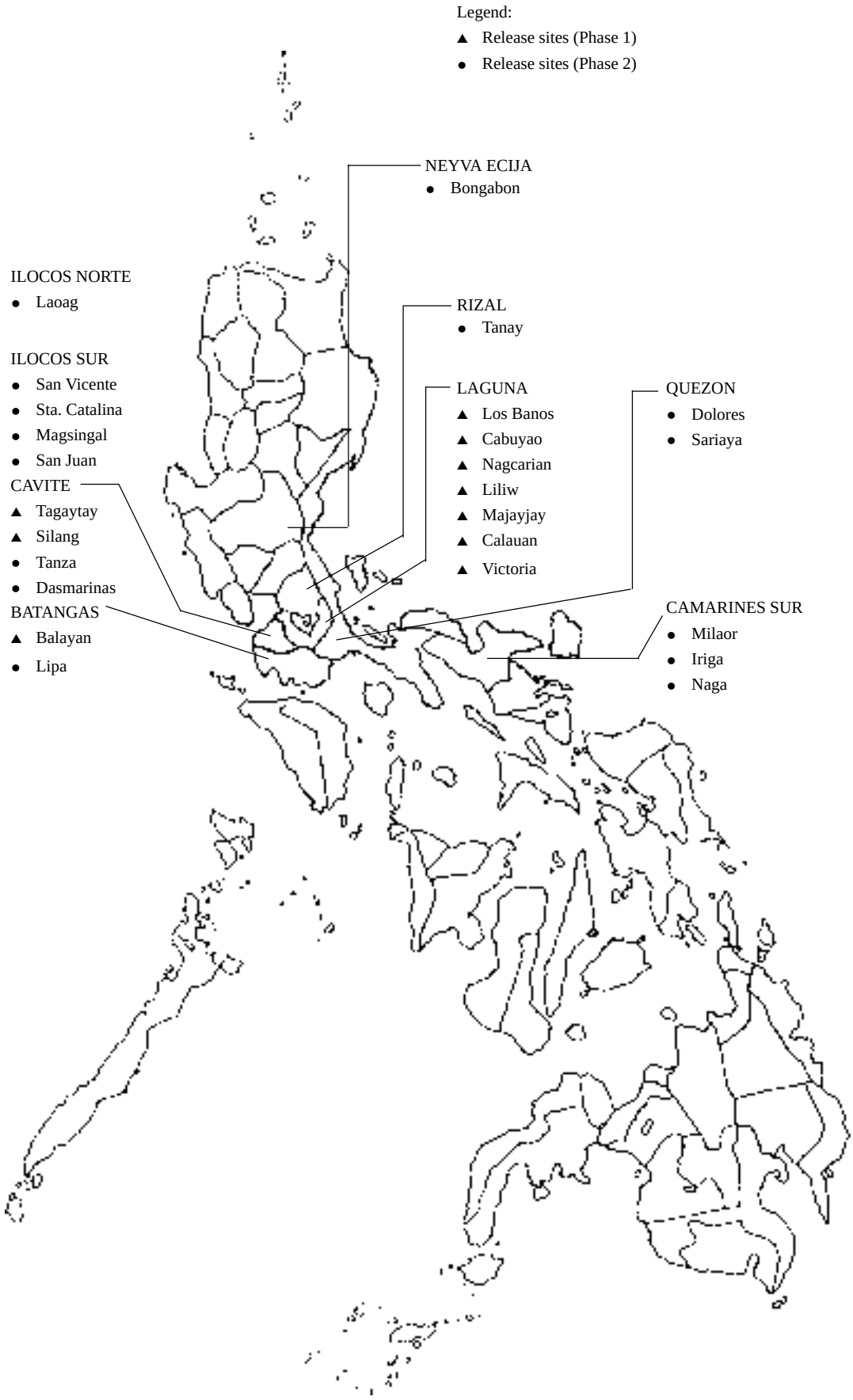


*Figure 1. Release sites of **Cotesia plutellae** Kurdj. in the Philippines*

The release of *Cotesia* reduced the DBM population below ETL in all release sites. In Quezon (1994) the average parasitism in IPM fields of 36% two weeks after the first release of *Cotesia* cocoons increased to 68% at the heading stage. The highest parasitism rate was 83%.

The rate of parasitism was low in IPM fields in all the release sites in Ilocos Sur (1995) during the first cropping especially if cooperators were not regularly visited by the agricultural technicians. The farmers tend to spray both the IPM and FCP fields; less than 5 DBM larvae/plant were noted in both fields. In the second cropping, 55% of the original cooperators planted cabbage and adopted the technology. The average parasitism and the number of *Cotesia* cocoons/ 50 plants in the IPM managed fields were 68% and 91 cocoons, respectively, at heading stage. The highest parasitism rate was 86%. The farmers learned to monitor the DBM population using ETL as basis for insecticide application. In fact, farmers sprayed their FCP fields with microbial insecticides. Thus, *Cotesia* cocoons developed and an average parasitism of 50% was noted.

In Ilocos Norte (1996) during the first cropping the farmers tend to spray the IPM and FCP fields. But the application in the former was 3–5 times with microbial insecticides while the latter 5–17 times with fenvalerate and methamidophos. DBM population was below ETL of 5 larvae/plant. In spite of the sprayings, *Cotesia* cocoons developed (18/50 plants); 15% average parasitism at heading stage was noted in the IPM field while no parasitism in FCP. In the second cropping, the average parasitism of 16% and 34 *Cotesia* cocoons/50 plants were noted at heading stage in IPM fields.

Establishment of *C. plutellae*

C. plutellae is established in release sites in Laguna (Liliw, Nagcarlan and Cabuyao), 5–6 years after last release with parasitism ranging from 30 to 88% *(Table 1)*. In Batangas, *C. plutellae* was already established 24 months after the last release in 1991 with parasitism of 51% (Morallo-Rejesus *et al.*, 1994) but when visited in April 1994, the farmer shifted to other crops.

C. plutellae is also established in the release sites in Quezon (Dolores, Sariaya), 1.2–2.6 years after last release with parasitism ranging from 25 to 50%.

In Ilocos Sur, no cocoons or parasitized larvae were collected 8–12 months after the last release in 1995 but adults of *C. plutellae* were observed in the field. This is because crucifers are grown after rice in this province.

Establishment of *C. plutellae* is slow in other release sites in some municipalities due to intermittent plantings, spraying of non-selective insecticides, and shifting to crops other than crucifers by the farmers.

Farmers' attitudes

The farmer's attitude based on six years experiences in transferring the *Cotesia*-based IPM in lowland crucifers could be summarized as follows:

- Initially, few farmers would like to participate in "on-farm" demonstration of the IPM technology. Many are skeptical on the effectiveness of the friendly insects and resort to a "wait and see attitude". In San Vicente, Sta. Catalina (Ilocos Sur) and Bongabong (Nueva Ecija), more farmers volunteered when informed that the parasitoids and microbial insecticides will be provided free and that the differences in yield of IPM (if lower) from FCP due to DBM infestation, will be compensated by AVNET in cash or in kind.

Table 1. Establishment of *C. plutellae* in release sites of Laguna and Quezon

Location	Date of monitoring	Ave. DBM population	No. of *Cotesia* cooons/50 plants	Percent Parasitism	Last date of release
LAGUNA					
Mamatid (Cabuyao)	June 93	2.38	36	47	Oct. 91
	Mar. 95	1.15	19	30	Oct. 91
Novaliches (Liliw)	May 93	4.96	52	58	Aug. 90
	May 96	0.62	93	88	Aug. 90
Bucal (Nagcarlan)	Mar. 93	3.92	43	69	Feb. 90
	May 96	0.32	45	65	Feb. 90
QUEZON					
Kinabuhayan	Jan. 94	1.94	15	40	Apr. 93
(Dolores)	Jan. 95	0.72	19	40	Apr. 94
	Aug. 95	0.95	11	50	Apr. 94
	Mar. 96	1.20	8	25	Apr. 94
Bangkong Kahoy					
(Dolores)	Apr. 96	0.54	16	46	Oct. 93
Mamala Uno					
(Sariaya)	Feb. 95	1.12	10	50	Nov. 94
	Oct. 95	1.10	9	52	Nov. 94
	Mar. 96	1.12	22	46	Nov. 94

- Most of the farmers who participated in the on-farm demo were progressive farmers, farmer leaders and high school graduates. Some progressive farmers with large farms and capital tend to be skeptical about the technology. After all, they could afford the cost of frequent sprayings.
- Farmer cooperators regularly visited and guided by AVNET personnel and/or researchers from CSU implemented the IPM-DBM technology properly in their farms and were able to encourage other farmers to adopt the technology.
- Farmer cooperators not regularly visited and assisted by the AVNET personnel tended to spray the designated IPM-managed fields. Without assistance from the trainers, farmers lack the confidence to rely on parasitoids for fear of crop losses.
- The farmer-participated demo farms were very successful (70–100%) in municipalities where the municipal agricultural officers (MAO's) and agricultural technicians (AT's) joined hands with the AVNET project personnel and or SCU personnel in visiting and guiding the farmers. Those without regular assistance only 30% of the cooperators were successful.

Demo farms were discontinued in areas (Cavite, Nueva Ecija, Rizal, Camarines Sur) where MAO's/AT's could not visit on a weekly basis at least during the initial introduction of the IPM technology. With limited manpower and too many sites to cover, the release sites could not be monitored regularly by AVNET personnel.

Impact of IPM technology

1. The philosophy of IPM and the parasitoid-based technology for DBM was imparted to 1 134 farmers and 176 researchers and technicians (trainers) of 31 municipalities and 9 provinces through trainings and lectures.
2. The cooperators' adoption of the technology resulted to:
   - Increased yield ranging from 18.65 to 72.02t/ha and reduced production cost ranging from US$1 04.16 to 3 902.43/ha, 2–4% lower than the farmer control practice (FCP) *(Figures 2–7)*. This resulted in the 5 to 78% increase net income than FCP ranging from US$2 781.25–10 984.48/ha.
   - Reduced insecticidal sprayings from 15–36 times to 1–9 times before introduction of technology and spraying with selective insecticides based on ETL.
3. The farmer-participated demo farms set-up in 49 farmers' fields in 21 barangays of 14 municipalities in 7 provinces served as a show window.
   - It convinced the cooperators, researchers, LGU personnel and other farmers of the effectiveness of the technology.
   - Farmers learned to monitor for the ETL as basis for deciding when to supplement with insecticidal sprays.
   - It influenced other farmers to adopt the technology or at least reduce the frequency

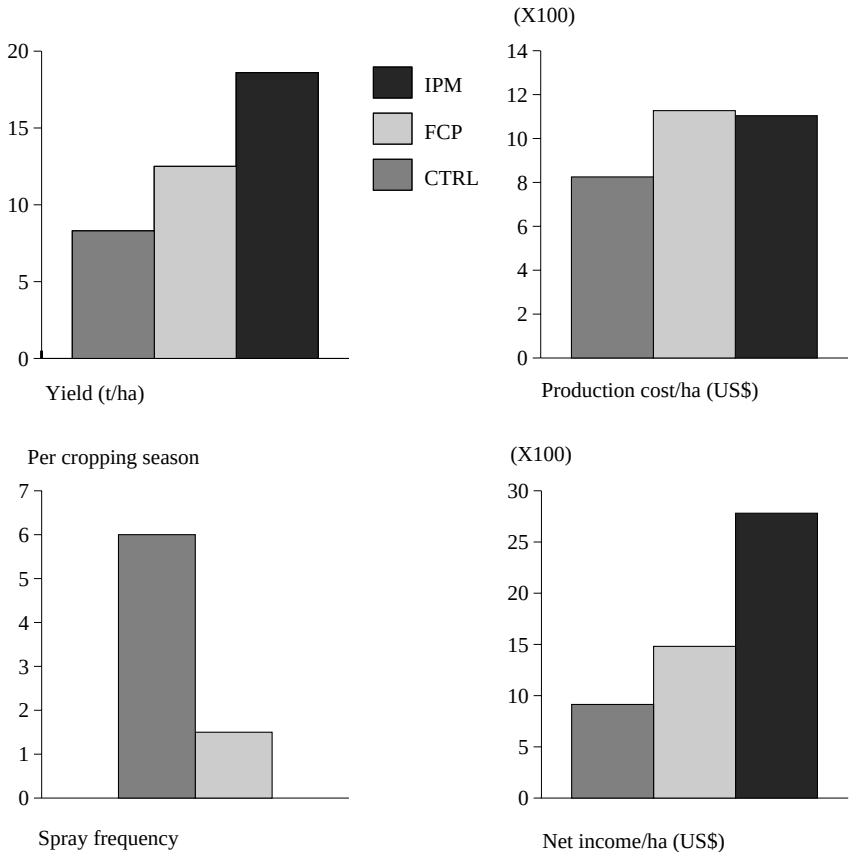


Figure 2. Yield and income of cabbage in IPM and FCP fields in Kinabuhayan, Dolores, Quezon, Philippines

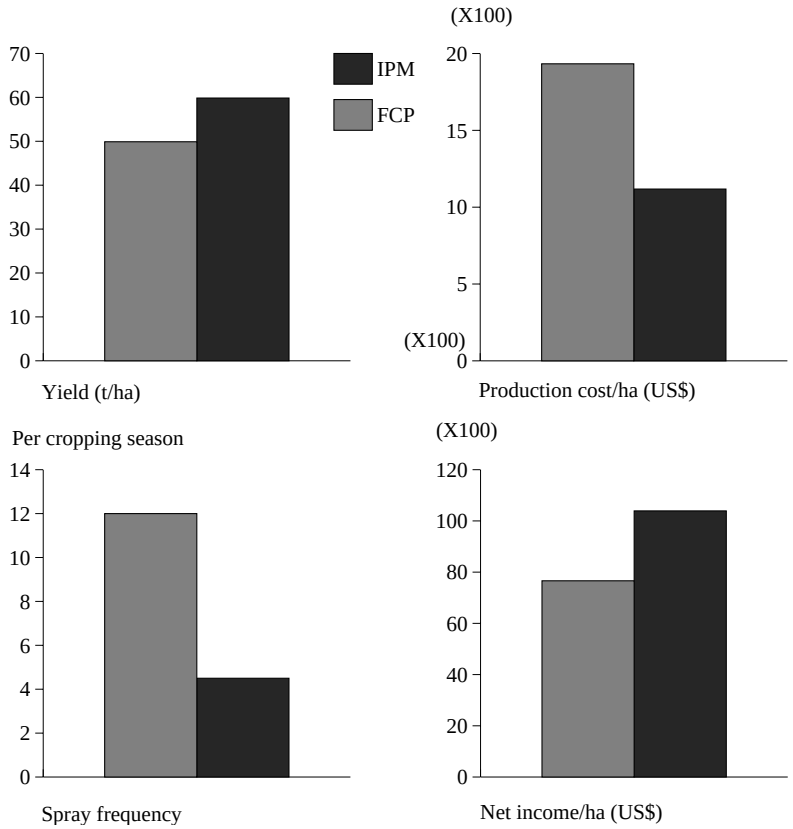


Figure 3. Yield and income of cabbage in IPM and FCP fields in Sta. Catalina, Ilocos Sur, Philippines

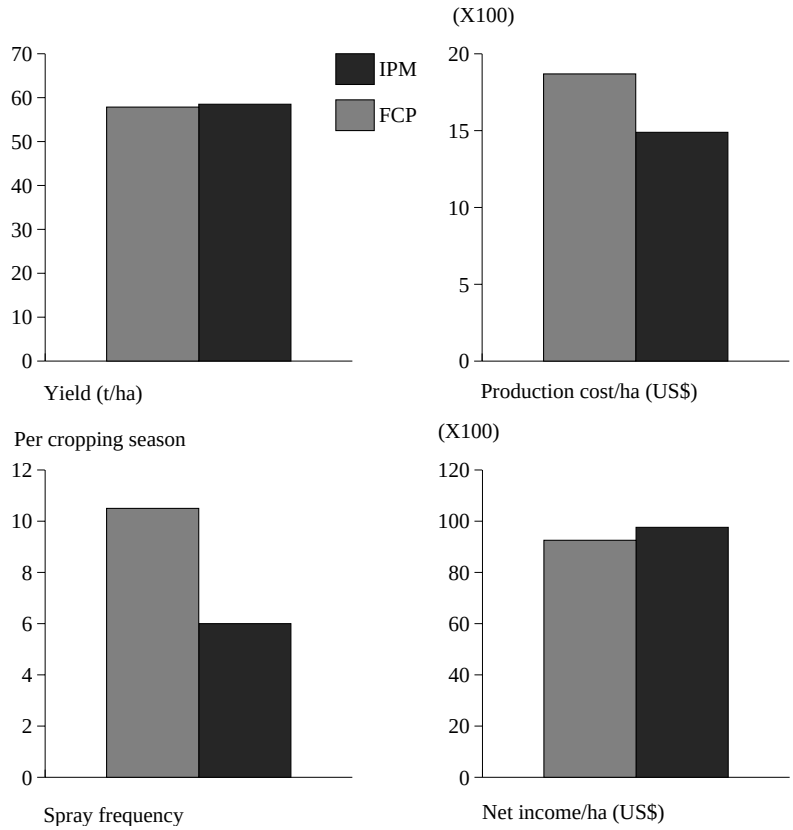


Figure 4. Yield and income of cabbage in IPM and FCP fields in San Vicente, Ilocos Sur, Philippines

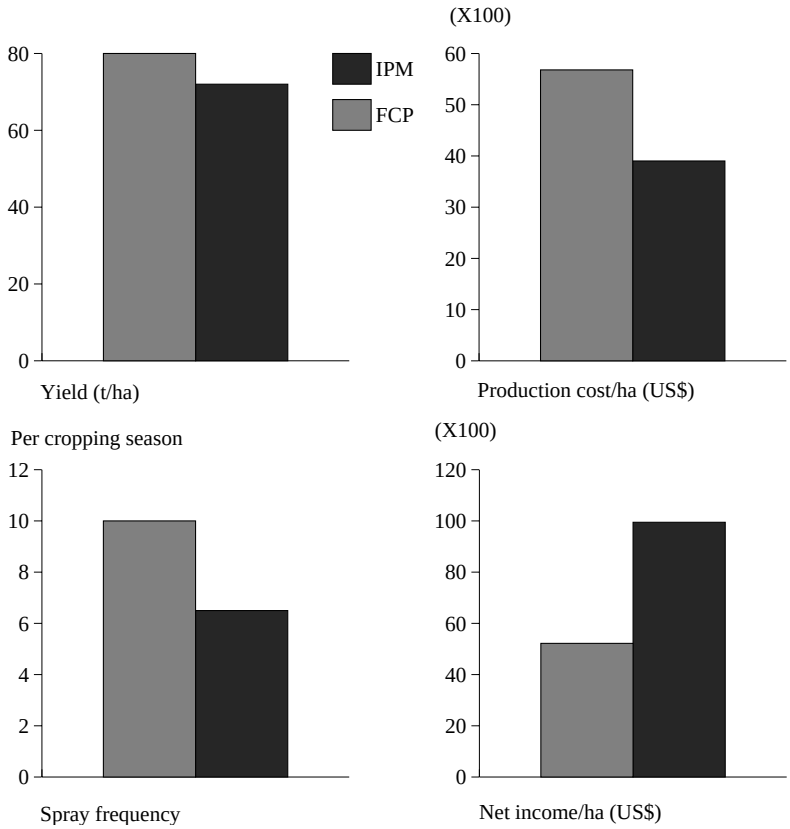


Figure 5. Yield and income of cabbage in IPM and FCP fields in San Juan, Ilocos Sur, Philippines

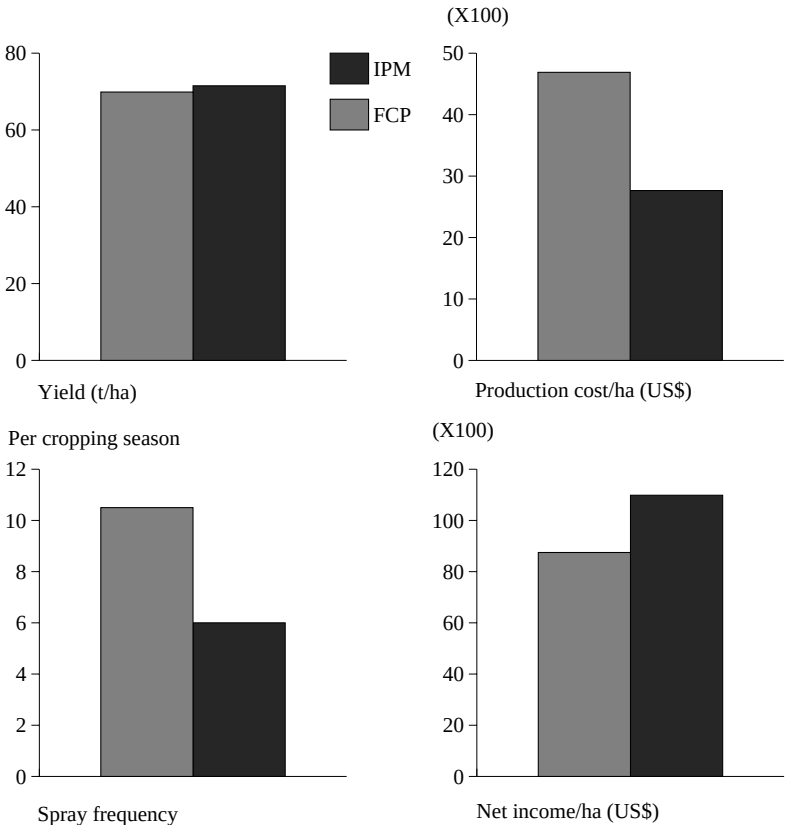


Figure 6. Yield and income of cabbage in IPM and FCP fields in Magsingal, Ilocos Sur, Philippines

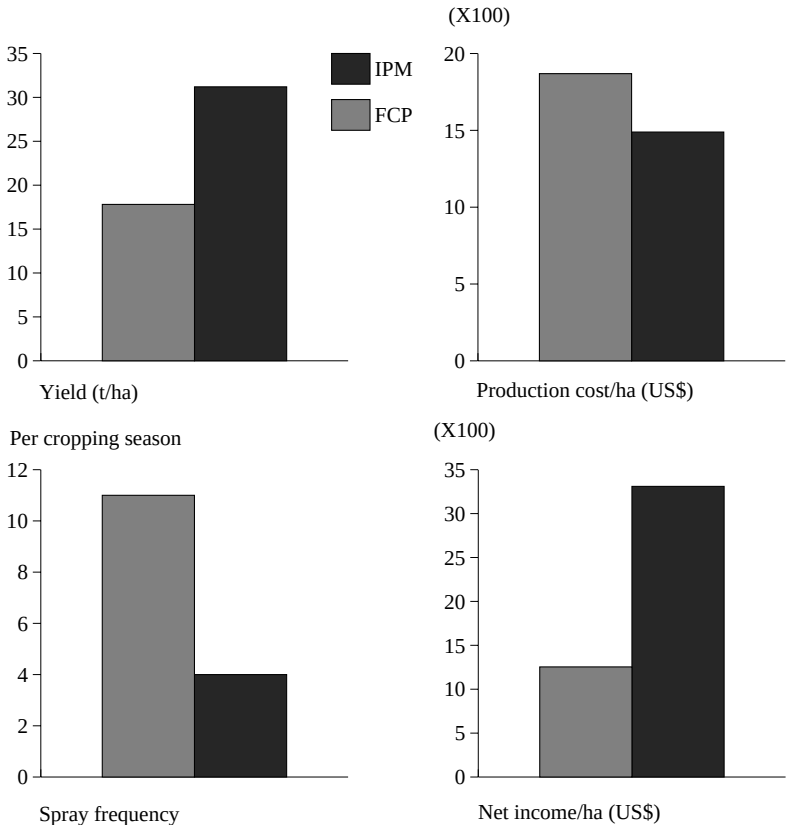


Figure 7. Yield and income of cabbage in IPM and FCP fields in Laoag City, Ilocos Norte, Philippines

of sprayings from 15–36 times to 8–15 times, and the use of selective insecticides especially the microbials.

- Increased awareness by farmers of the role of natural enemies in IPM and conserving them by spraying only when necessary and with selective insecticides.
- Increasing farmers' awareness on the hazards of mixing insecticides, using non-selective and unrecommended insecticides, and using dosages beyond the recommended rates.

4. *C. plutellae* is established in release sites, and present in neighbouring crucifer areas resulting to relatively low DBM population.
5. The networking with local government units led to increased awareness of government officials on the role of the friendly insects in the pest management. Thus they supported the initiatives of trainers from LGU, DA and SCUs in promoting the IPM-DBM technology.
6. The transfer of *Cotesia*-based IPM, subsequently, reduced the hazards associated with consumption of pesticide-ridden vegetables and the pollution of the environment.

Recommendations

The Department of Agriculture and LGUs should ensure sustainable institutional support for the transfer of the technology and marketing of the vegetables to many farmers especially unlettered resource-poor farmers.

Since DBM is not the only destructive pest of crucifers, research on the development of IPM for other major pests of crucifers should be supported by the national government and or external organizations.

The AVNET project with AVRDC and other countries, with local coordination by PCARRD, has been fruitful and beneficial. Therefore, linkages with external organization/institutions that seek to rationalize pest management practices in crucifers and other crops should be given top priority.

Acknowledgement

Authors wish to acknowledge the financial assistance of Asian Development Bank (ADB) through AVRDC and the counterpart local funding from the national government through PCARRD. The technical assistance from AVRDC especially Dr. N.S. Talekar in the implementation of the project in IPM is greatly appreciated.

Literature Cited

AVRDC (1990, 1989). Progress Report. Asian Vegetable Research and Development Center. Shanhua, Taiwan, R.O.C. 351 pp.

Barroga, S. F. (1967). Screening of chemicals for the control of diamondback moth (*Plutella xylostella*) attacking cabbage. *Ann. Rpt. Bu. Plt. Ind. Fy.* 1966–67.25 pp.

Barroga, S. F. and B. Morallo-Rejesus, (1975). A survey of diamondback moth *(Plutella xylostella)* population for resistance to insecticides in the Philippines. *The Phil. J. Plant Ind.* ***40–41:*** 1–14.

Cadapan, E. P. and B. P. Gabriel (1972). Field evaluation of Dipel and other commercial *Bacillus thuringiensis* in comparison with standard insecticides used in the control of diamondback moth, *Plutella xylostella* L. and other insect pests affecting crucifers. UPCA Ann. Rpt. 1972–1973. 15 pp.

Cardona, E. V. (1986). Study on the effective concentration of Nomolt 5SC and Thuricide WP against DBM infesting head cabbage. Terminal Rpt. Benguet State University (BSU). 10 pp.

Ebuenga, C. (1992). Detection of resistance of diamondback moth (*Plutella xylostella* [linnaeus]) to twelve insecticides. M.S. Thesis, Dept. of Entomology, UPLB, 84 pp.

Lim, G.S. (1986). Biological control of diamondback moth. *In* Talekar, N.S. and Griggs, T.D. (eds.), Diamondback Moth Management. Proc. 1st International Workshop, AVRDC, Shanhua, Taiwan. p. 159–171.

Morallo-Rejesus, B. and A. S. Sayaboc, (1990). Management of diamondback moth with *Cotesia plutellae*: Prospect in the Philippines. *In:* Talekar, N.S. (ed.). Management of DBM and Other Crucifers Pests. Proc. 2nd International Workshop, Tainan, Taiwan, AVRDC Publ. # 92–368. p. 279–286.

Morallo-Rejesus, B., A. S. Sayaboc, G. P. Fajardo and J.E. Eusebio (1994). Introduction and field releases of larval parasitoid *Cotesia plutellae* (Kurdj.) for the management of diamondback moth, *Plutella xylostella* (L.) of crucifers in the Philippines. *Phil. Agric.* ***77(4):*** 481–499.

Ooi, P. and G. S. Lim, (1989). Introduction of exotic parasitoids to control the diamondback moth in Malaysia. *J. Plant Prot. Trop.* ***6:*** 103–111.

Sastrosiswojo, S. and G. Sastrodihardjo, (1986). Status of biological control of diamondback moth by introduction of parasitoid *Diadegma eucerophaga* in Indonesia. *In:* N.S. Talekar and Griggs, T. D. (eds.) Diamondback Moth Management. Proc. First International Workshop, AVRDC, Shanhua, Taiwan. p. 50–54.

Talekar, N. S. (1990). Development of an integrated pest management program for the control of diamondback moth on cruciferous vegetables. *In* Opena, R.T. and Kyomo, M.L. (eds.) Veg. Res. and Dev. in SADCC countries. AVRDC, Shanhua, Taiwan. p. 147–157.

Talekar, N. S., Yang, S. C. and S. T. Lee, (1992). Introduction of *Diadegma semiclausum* to control diamondback moth in Taiwan. In N.S. Talekar (ed.). Diamondback Moth and Other Cruciferous Pests; Proc. 2nd Int'l Workshop. Tainan, Taiwan, AVRDC Publ. No. 92–368. p. 263–270.

Introduction and evaluation of *Cotesia rubecula*, a parasitoid of *Pieris rapae* in New Zealand

P.J. Cameron and G.P. Walker

New Zealand Institute for Crop & Food Research Ltd, Private Bag 92169, Auckland, New Zealand.

Abstract

The host specificity of *Cotesia rubecula* (Marshall) was examined through literature searches, behavioural and ecological observations, and host specificity tests of a South Australian population. The results led to the importation and testing of *C. rubecula* in quarantine in New Zealand where its specificity to the genus *Pieris* was confirmed. Introduction of the parasitoid was approved and the species was first released in New Zealand for control of *Pieris rapae* (L.) in December 1993. It has now overwintered successfully and has parasitised 71–97% of larvae at study sites. Three paired comparisons of sites with and without the parasitoid showed that *C. rubecula* significantly reduced the survival of *P. rapae* to 5th instar, and also reduced parasitism by *Cotesia glomerata* (L.).

Key words: *Cotesia rubecula*, *Pieris rapae*, host specificity, introduction, impact.

Introduction

Pieris rapae (L.) (Lepidoptera: Pieridae), small white butterfly, is second only in importance to *Plutella xylostella* (L.), (Lepidoptera: Plutellidae), diamondback moth, as a pest of vegetable brassicas in New Zealand. The existing larval parasitoid, *Cotesia glomerata* (L.) is not well synchronised with its host and often provides insufficient control in the summer (Beck and Cameron, 1992). The introduction of *Cotesia rubecula* (Marshall) (Braconidae: Microgastrinae) was therefore proposed. Although *C. rubecula* partially displaces *C. glomerata* (Parker and Pinnell, 1972), the overall efficacy of parasitism is improved by the reduced feeding of larvae parasitised by *C. rubecula* (Parker and Pinnell, 1973). A further factor favouring approval of *C. rubecula* for introduction into New Zealand was its reputed specificity to the genus *Pieris*. Richards (1940) confirmed that *C. rubecula* is almost specific to *P. rapae* and noted that exceptionally it will attack *Pieris brassicae* (L.) In his description of *C. rubecula*, Wilkinson (1945) noted that other than *Pieris* spp., the only other recorded host was *P. xylostella*. Mustata (1992) also recorded *P. xylostella* as a rare host in Moldavia, although in Australia, *C. rubecula* has not been recovered from this species (Austin and Dangerfield, 1992; Goodwin, 1979). Approval for release in New Zealand required the verification of this specificity, which is documented in this paper. The second part of this paper reports on the release, dispersal and impact of *C. rubecula*.

Methods

Host specificity

Initial information on the host specificity of *C. rubecula* was based on the field collections of Dr M.A. Keller and G.J. Baker of *P. xylostella* and *Anaphaeis java* (Sparman) (Papilionoidea: Pieridae) in the Adelaide region of South Australia. In addition, *Bassaris itea* F. (Papilionoidea: Nymphalidae) larvae were collected (by PJC) from this region in 1992 and 1994. The acceptability of these three species and *P. rapae* to *C. rubecula* was also compared in flight tunnel tests at the University of Adelaide using the methods of Keller (1990). *A. java* was presented on *Capparis mitchelli* (Capparaceae), *B. itea* on nettle (*Urtica dioica*), and *P. xylostella* and *P. rapae* on cabbage. The preference of *C. rubecula* females was tested by presenting 3–6 larvae per leaf of each test species as a choice compared with *P. rapae* larvae. The choice by female parasitoids between plants, and any subsequent oviposition into larvae, was noted for each of five tests for each combination.

Confirmatory testing of oviposition responses was also carried out in quarantine at Crop & Food Research in Auckland, New Zealand. Individual mated female parasitoids were exposed in 10 x 2.5 cm glass tubes to single larvae of alternate species, each for a period of 5 minutes. Ten to 24 larvae, 3–10 mm in size were tested for each species. Oviposition responses were recorded and when any response occurred the larva was removed and reared individually on its usual host plant until parasitism was confirmed or the larvae pupated normally. To confirm that females were capable of oviposition, every second or third test larva was *P. rapae*. Larvae were either tested with no plant matter, or with their usual host plant, or after confinement with cabbage. In experiments over longer exposure periods, two mated female parasitoids were placed in 450 ml vented plastic containers for 16 h with 10 larvae on their original host plant, or cabbage. Voucher specimens were deposited in the New Zealand Arthropod Collection at Landcare Research in Auckland.

Release and recovery

Cultures of *C. rubecula* in New Zealand were based on four shipments totalling 383 *C. rubecula* cocoons in 1993/94 (Cameron *et al.* 1995) and one shipment of 85 cocoons in 1994/95, all supplied by Dr M.A.

Keller of the University of Adelaide in South Australia. Females from the source culture were mated with field-collected males in Australia to maintain genetic variability in the shipments. In New Zealand, all importations were reared separately for at least two generations to ensure each shipment contributed parasitoids for release. A disease-free culture of *P. rapae* was established on cabbage to provide small larvae for parasitism. The resulting cocoons of *C. rubecula* were harvested for release, placed in 20 x 5 cm cardboard tubes (100–200/tube) with a small exit hole at one end, and supplied with honey-agar-sugar as food for emerging adults. These release containers were placed in small shelters at canopy height in brassica field sites where larvae of *P. rapae* were present. Sites were located at research stations or organic gardens where a succession of unsprayed brassica crops were planted. A total of 9456 parasitoids were released in 1993/94, and 20167 in 1994/95 *(Table 1)*.

Release sites near Auckland were monitored every 1–2 weeks, and parasitism was assessed by collecting and rearing a minimum of 40 larvae. To confirm over-wintering, surveys were undertaken the following spring or summer after initial releases. Estimates of levels of parasitism were also gained from these collections, and parasitism by *C. glomerata* and the occurrence of hyperparasitoids were recorded from all collections. The dispersal of *C. rubecula* from a release site was monitored weekly from 30 January to 7 March 1996 in the Pukekohe (South Auckland) vegetable-growing region by placing trap plants (cabbages with 30–50 small larvae) at approximately 0.5–1.0 km spacings out from the release site. Plants were placed in pairs (about 10 m apart) at the edge of vegetable brassica crops or near road-side brassica weeds, left in the field for two days, and then collected. Larvae were then reared in the laboratory to determine the extent of parasitism. If *C. rubecula* was recovered, plants were located further away from the original site on the next test occasion until no further parasitism was detected.

Impact

The impact of *C. rubecula* on *P. rapae* was evaluated near Auckland on three occasions at pairs of experimental sites separated by 2–10 km. One site in each pair was an earlier release site for *C. rubecula* and the other site was in the same growing region, but without the new parasitoid. This comparison was performed on cabbage in the 1993/94 and 1994/95 summer seasons, and on broccoli in the 1995/96 summer. The sites consisted of at least 400 plants of similar age that had received no insecticide applications. Size distribution of *P. rapae* larvae was assessed by recording the number of larvae in each instar in weekly samples from 20–40 randomly selected plants. Rates of parasitism were monitored by collecting and rearing the first 30–60 large 1st to 3rd instar larvae encountered during this weekly sampling. Percent parasitism was calculated as the number of parasitised larvae compared with the number of survivors plus parasitised larvae.

Results and Discussion

Host specificity

In the Adelaide region of South Australia, investigations of host specificity were focused on near relatives of *P. rapae*. The closest relative of *P. rapae* that occurred close to mixed cropping areas where *C. rubecula* was present was the pierid, *A. java*. These larvae were common on *C. mitchelli* in the grounds of the Waite Campus of the University of Adelaide, but were not parasitised by *C. rubecula* (Austin and Dangerfield, 1992; M.A. Keller, pers. comm.). *B. itea*, the yellow admiral, occurs on nettle *(U. dioica)* in both Australia and New Zealand. As New Zealand has few attractive butterflies there is interest in ensuring its conservation. Approximately 132 larvae of this species were collected from six locations around Adelaide over two summers. None were parasitised. Previous extensive collections of *P. xylostella* from vegetable and wild brassicas by M.A. Keller and G.J. Baker confirmed that *C. rubecula* was not a parasitoid of this species in the Adelaide region.

In flight tunnel experiments, *C. rubecula* was attracted to and oviposited in *P. rapae*, but females were not attracted to either *A. java* or *B. itea*. Any females that alighted on *Capparis/A. java* or nettle/*B. itea* immediately took flight and often moved to cabbage. In the comparison of *P. rapae* with *P. xylostella*, female parasitoids flew equally to either plant, but oviposition responses were directed only at *P. rapae*. These observations are consistent with those of Agelopoulos and Keller (1994).

Table 1. Releases and recoveries of *Cotesia rubecula* to April 1996, showing overwintering success and peak parasitism

Region	Release sites	First release	Number released	Recovery sites	Over-wintered (sites)	Peak parasitism (%)
Northland	11	Feb. 1994	5 328	4	2	61
Auckland	14	Dec. 1993	13 227	7	5	97
Feilding	3	Dec. 1994	3 374	3	2	14
Levin	3	Mar. 1994	2 711	3	1	88
Canterbury	2	Mar. 1994	3 267	2	1	71
Gore	1	Mar. 1994	1 816	1	0	52
Total	34		29 723	20	11	

Following the apparent specificity suggested by these tests, *C. rubecula* was imported into quarantine in New Zealand for further oviposition tests with relatives of *P. rapae* and other Lepidoptera found on brassicas, including native species *(Table 2)*. These tests attempted to force oviposition, or ovipositional errors, by artificially associating the parasitoid and test larvae. In addition, test larvae occasionally found on brassicas in the field were fed on cabbage prior to testing, thus generating potential oviposition stimuli in the form of frass volatiles. Oviposition was obtained only with *P. rapae*, but occasional oviposition-like probing was observed with *Graphania mutans* Walker (Noctuidae) and *P. xylostella (Table 2)*. Rearing and dissection of probed individuals detected no eggs or larvae, and no parasitoid cocoons were formed. All parasitoid stages were detected in *P. rapae* control insects. Choice experiments with *P. rapae* and *G. mutans* or *P. xylostella* showed that parasitoids would walk over the alternate species to selectively oviposit in adjacent *P. rapae*.

It was concluded that *C. rubecula* from the Australian source was specific to the genus *Pieris*. As there are no other Pieridae in New Zealand, the parasitoid was considered to be safe to import. Following public consultation, permission to release was obtained and the parasitoid was first released in December 1993.

Establishment and dispersal

At frequently sampled experimental sites in the Auckland region, parasitised larvae were recovered within 2–4 weeks of release, and in the first season parasitism reached 25–97% in five geographic regions (Cameron *et al.*, 1995). Overwinter survival was not recorded in all regions after one season of releases, but after two seasons of releases the parasitoid was considered to be established in all but one region *(Table 1)*. No geographic or climatic limitations to the establishment of the parasitoid were detected. The few failures to persist were attributed to low host populations rather than any biological limitations of *C. rubecula*.

Dispersal from release sites has so far been comparatively slow. In the first four months following release in the Pukekohe vegetable-growing region, parasitoids were detected 2.1 km from the release point. By March 1996, two years and three months after release, *C. rubecula* had spread approximately 12 km. By contrast, *Cotesia kazak* Telenga (Braconidae: Microgastrinae), a parasitoid of *Helicoverpa armigera* Hubner (Noctuidae), spread approximately 100 km in one year from the same Pukekohe release site (Cameron and Valentine, 1985).

Impact

The release of *C. rubecula* at experimental sites and its relatively slow dispersal allowed the comparison of sites with and without natural parasitoid populations. Weekly sampling of paired sites showed that as populations of *P. rapae* developed on brassica crops, fewer larvae survived to reach 5th instar where *C. rubecula* was present (*Figures 1a* and *b)*. In the cabbage trial at Kumeu in 1994/95, parasitism at the release site ranged from 71 to 77%. Just prior to harvest, no 5th instar larvae were found on 20 sample plants at the parasitoid release site, whereas an average of 1.95 large 5th instar larvae/plant were found at the site without *C. rubecula (Table 3)*. A comparison of the instar distribution of these larvae *(Fig. 1a)* showed that parasitism caused a high level of mortality in 4th instar *P. rapae*.

Similar results were obtained in the broccoli trial in 1995/96 where parasitism at the release site ranged from 71 to 93% *(Figure 2a)*. Although *P. rapae* populations were more dense than at the control site, very few larvae survived to enter the 5th instar. At the control site, *C. rubecula* was absent until one individual appeared in each of the last two sampling occasions, demonstrating successful dispersal from the release site *(Figure 2b)*. As the population at the control site developed, large 5th instar larvae became common on the plants. Just prior to harvest , 80% of the broccoli heads were infested with an average of 2 large larvae/plant. Comparison of the instar distribution of larvae at sites with and without *C. rubecula* showed high

Table 2. Oviposition responses by *C. rubecula* to test larvae related to *P. rapae*, and larvae associated with brassicas

Test larvae	Possible oviposition	Parasitoid development	Number of observations
Pieris rapae (L.) (Papilionoidea: Pieridae)	149	145	149
Related species			
Bassaris itea F. (Papilionoidea: Nymphalidae), native	0	–	18
Danaus plexippus L. (Papilionoidea: Nymphalidae)	0	–	20
Zizina labradus Godart (Papilionoidea: Lycaenidae), native	0	–	18
Species from brassicas			
Agrotis ipsilon (Hufnagel) (Noctuidae)	0	–	18
Epiphyas postvittana Walker (Tortricidae)	0	–	18
Graphania mutans Walker (Noctuidae), native	7	0	68
Helicoverpa armigera (Hubner) (Noctuidae)	0	–	24
Phrissogonus laticostatus Walker (Geometridae)	0	–	10
Plutella xylostella (L.) (Plutellidae)	3	0	76
Thysanoplusia orichalcea F. (Noctuidae)	0	–	23

Table 3. Mean number of 5th instar larvae/plant (n=20), two to three weeks prior to harvest

Site, year and crop	Mean number/plant (±SE)	
	With *C. rubecula*	Without *C. rubecula*
Pukekohe, 1993/94, cabbage	0.05 ± 0.05	1.65 ± 0.35
Kumeu, 1994/95, cabbage	0	1.95 ± 0.37
Pukekohe, 1995/96, broccoli	0.25 ± 0.12	2.05 ± 0.42

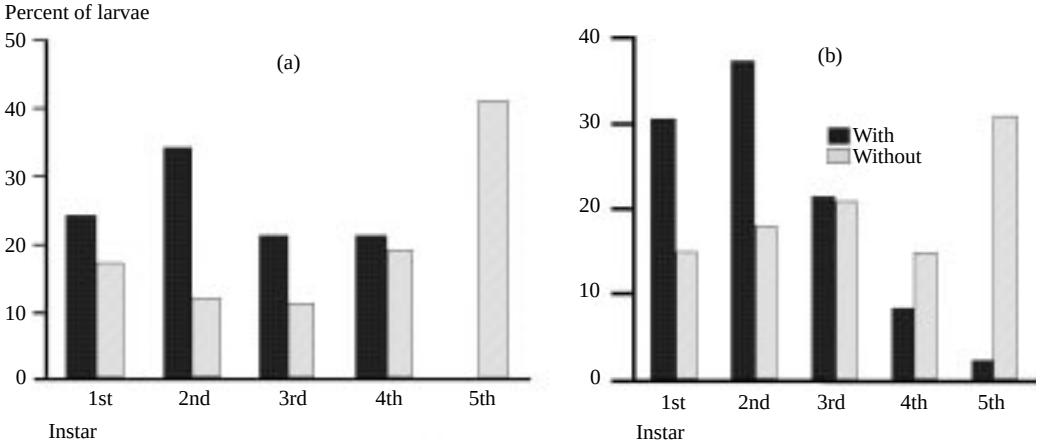


Figure 1. Instar distribution of P. rapae populations with or without C. rubecula at (a) paired cabbage sites at Kumeu, 1994/95, and (b) paired broccoli sites at Pukekohe, 1995/96

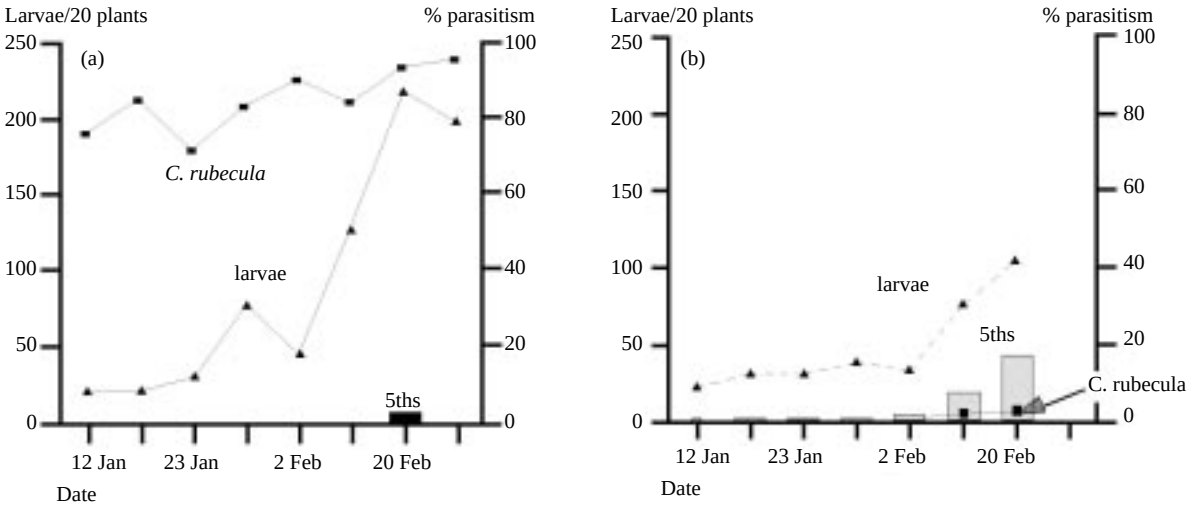


Figure 2. Development of P. rapae larval populations and 5th instar larvae, and parasitism by C. rubecula at (a) a previous release site for C. rubecula, and (b) a control site; Pukekohe, 1995/96

mortality from parasitoids emerging from 4th instar larvae *(Figure 1b)*.

Estimates of parasitism by *C. rubecula* and the multiple parasitoid *C. glomerata* showed the dominance of *C. rubecula*. Without *C. rubecula* at the broccoli control site in 1996, parasitism rates for *C. glomerata* reached 50% *(Figure 3a)*. Where both parasitoids were present, *C. glomerata* parasitism remained less than 10% *(Figure 3b)*. This finding is consistent with field observations by Parker and Pinnell (1972) and laboratory experiments by Laing and Corrigan (1987). Parker and Pinnell (1973) also demonstrated that partial displacement of *C. glomerata* was not detrimental to contol of *P. rapae* because larvae parasitised by *C. rubecula* are killed in the 4th instar and eat significantly less than those parasitised by *C. glomerata*.

Estimates of the infestation of plants by 5th instar larvae provided a summary of the impact of *C. rubecula* in all the experimental comparisons *(Table 3)*. As the paired sites were planted at similar dates and were within 10 km of each other, the seasonal or climatic differences between sites were minimised. All sites were free of insecticide applications, therefore natural enemies other than *C. rubecula* were also abundant and contributed to mortality. However, consistent differences between the size distribution of *P. rapae* larvae with and without *C. rubecula*, together with the high rates of parasitism, indicated that *C. rubecula* was a major factor in reducing the populations of large, damaging *P. rapae* larvae. Although these differences were reflected in reduced crop damage by *P. rapae* at sites where *C. rubecula* was present, damage from *P. xylostella* continued to

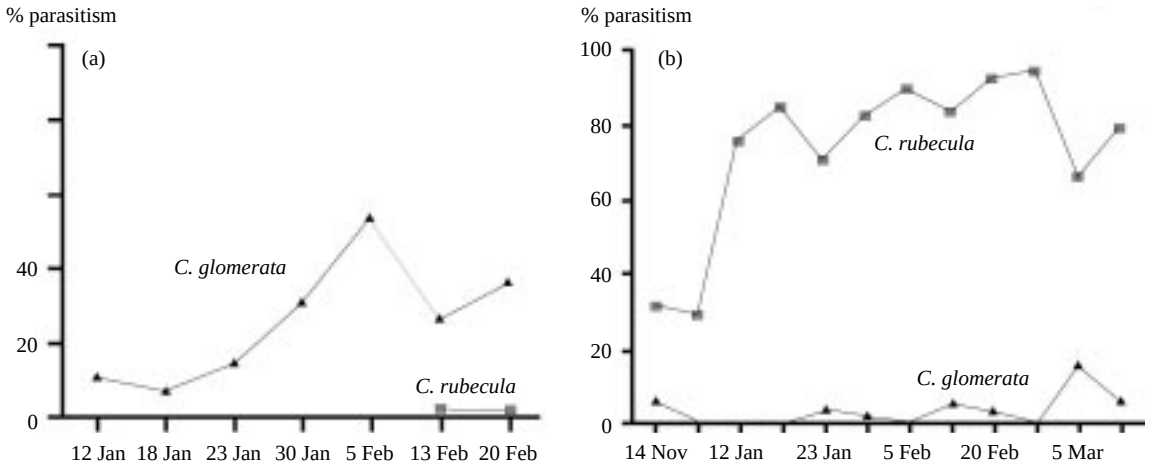


Figure 3. Parasitism of P. rapae by C. rubecula and C. glomerata at (a) a previous release site for C. rubecula and (b) a control site; Pukekohe, 1995/96

be the dominant problem at two of the three sites. Research to improve biological control of *P. xylostella* is the subject of an additional research programme also reported (see Cameron *et al.*, 1997) in these proceedings.

Acknowledgements

We thank Mike Keller for assistance with collections and testing in Adelaide and, together with Greg Baker, for information from previous rearing records. Sarah Painter and Guy Penny provided valuable assistance with surveys, sampling and rearing.

References

Agerlopoulos, N. G. and Keller, M. A. (1994). Plant-natural enemy association in the tritrophic system *Cotesia rubecula-Pieris rape*-Brassicaceae (Cruciferae): II. Preference of *C. rubecula* for landing and searching. *Journal of Chemical Ecology* ***20:*** 1 735–1 748.

Austin, A. D. and Dangerfield, P. C. (1992). Synopsis of Australian Microgastrinae (Hymenoptera: Braconidae), with a key to genera and description of new taxa. *Invertebrate Taxonomy* ***6:*** 1–76.

Beck, N. G. and Cameron, P. J. (1992). Developing a reduced spray programme for brassicas in New Zealand. In *Diamondback moth and other crucifer pests* (ed. N.S. Talekar) pp. 341–350.

Cameron, P.J. and Valentine, E. W. (1985). Evaluation of *Cotesia kazak* (Hymenoptera: Braconidae), a parasite of *Heliothis armigera conferta* (Lepidoptera: Noctuidae) in New Zealand. *New Zealand Journal of Agricultural Research* ***28:*** 545–553.

Cameron, P. J., Walker, G. P., Keller, M. A. and Clearwater, J. R. (1997). Host specificity assessments of *Cotesia plutellae*, a parasitoid of diamondback moth. In: A. Sivapragasam *et al.* (eds.). Proceedings of the Third International Workshop on the Management of Diamondback moth and other Crucifer Pests, Malaysia.

Cameron, P. J., Walker, G. P. and Keller, M. A. (1995). Introduction of *Cotesia rubecula*, a parasitoid of white butterfly. *Proceedings of the 48th New Zealand Plant Protection Conference*: 345–347.

Goodwin, S. (1979). Changes in numbers in the parasitoid complex associated with the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera) in Victoria. *Australian Journal of Zoology* ***27:*** 981–989.

Keller, M. A. (1990). Responses of the parasitoid *Cotesia rubecula* to its host *Pieris rapae* in a flight tunnel. *Entomologia experimentalis et applicata* ***57:*** 243–249.

Laing, J.E. and Corrigan, J.E. (1987). Intrinsic competition between the gregarious parasite, *Cotesia glomeratus* and the solitary parasite, *Cotesia rubecula* (Hymenoptera: Braconidae) for their host *Artogeia rapae* (Lepidoptera: Pieridae). *Entomophaga* ***32:*** 493–501.

Mustata, G. (1992). Role of the parasitoid complex in limiting the population of diamondback moth in Moldavia, Romania. In *Diamondback moth and other crucifer pests* (ed. N.S. Talekar) pp. 203–212.

Parker, F. C. and Pinnell, R. E. (1972). Further studies on the biological control of *Pieris rapae* (L.) utilizing supplemental host and parasite releases. *Environmental Entomology* ***1:*** 150–157.

Parker, F. C. and Pinnell, R.E. (1973). Effect on food consumption of the imported cabbageworm when parasitised by two species of *Apanteles*. *Environmental Entomology* ***2:*** 216–219.

Richards, O. W. (1940). The biology of the small white butterfly *(Pieris rapae)*, with special reference to the factors controlling its abundance. *Journal of Animal Ecology* ***9:*** 243–288.

Wilkinson, D. S. (1945). Description of Palearctic species of *Apanteles* (Hymen., Braconidae). *Transactions of the Royal Entomological Society of London* ***95:*** 35–226.

Aphids on crucifers: multitrophic and selective insecticide interactions for enhanced control

Robert H.J. Verkerk and Denis J. Wright

Department of Biology, Imperial College at Silwood Park, Ascot, Berkshire SL5 7PY, U.K.

Abstract

Most studies on chemical and biological control of arthropod pests of agriculture do not consider the effects of partial host plant resistance. We present research using two partially resistant and a susceptible cultivar of common cabbage (*Brassica oleracea* var. *capitata* L.) and determining specific effects of interactions between the host plants, one or two common crucifer aphids (*Myzus persicae* Sulzer, a generalist species and *Brevicoryne brassicae* L., a crucifer specialist), selective insecticides (pirimicarb and a neem seed kernel extract) and a predator (the predatory gall midge, *Aphidoletes aphidimyza* Rondani). A pirimicarb dose equating to *c.* 15% of the recommended field concentration caused equivalent toxicity of *M. persicae* on a partially resistant cultivar compared with aphids treated with a three-fold greater dose and reared on the susceptible cultivar. Cultivar-mediated differences in mortality caused by a neem extract tested for systemic and translaminar activity (at the recommended concentration) were not apparent. In a laboratory-based tritrophic system including both species of aphid, three common cabbage cultivars and *A. aphidimyza*, cultivar effects were particularly pronounced in relation to predator survival from egg to adult, the survival rates being at least 2.5-fold greater on the susceptible cultivar (compared with the two partially resistant cultivars) with both aphid host species. Predator larval body length were also less on the partially resistant compared with susceptible cultivar with both aphid species as hosts. Considerable variation (between host plants/cultivars and sites) in *M. persicae* infestation intensity were noted in field studies in two different zones of the Cameron Highlands, Malaysia. The results are discussed in relation to ways in which compatibility between partial host plant resistance, selective insecticides and biological control may be manipulated and optimised in crucifer aphid management systems.

Key words: predator, tritrophic, host plant resistance, selective insecticide, neem

Introduction

Research on chemical and biological control of agricultural arthropod pests has, over the past half century, seldom taken into account variation mediated by different host plants (see Verkerk & Wright, 1996). Similarly, host plant resistance studies with phytophagous arthropods tend to have been carried out without considering interactions with chemical controls. Work on chemical control of particular target pests has generally involved measurement of pesticide-induced effects on each specific pest reared on a single cultivar of a key host plant species. Biological control has most often been studied as an interaction between specific predators or parasites and their prey, again without considering variation in the first trophic level. Most information on tritrophic (host plant - herbivore - natural enemy) interactions does not relate to agricultural pests (Price, 1986; Hare, 1992) and includes parasitoids as representatives of the third trophic level, rather than predators (Herzog & Funderbunk, 1985).

The present studies include the peach-potato aphid *Myzus persicae* Sulzer (Hemiptera: Aphididae), the cabbage aphid *Brevicoryne brassicae* L. (Hemiptera: Aphididae), two selective insecticides (pirimicarb and a neem seed kernel extract), the predatory gall midge *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae) and up to three cultivars of common cabbage (*Brassica oleracea* var. *capitata* L.). The studies attempt to provide preliminary data on the significance of partial plant resistance (in cvs Minicole and Ruby Ball, compared with the generally susceptible cv Derby Day) in this brassica-aphid pest management system.

Materials and Methods

All laboratory experiments were maintained in a controlled environment (20±2ºC; 65% RH, 16:8 L:D). All plants were grown in 7 cm square plastic pots with standard potting compost (Levingtons Multipurpose Compost®, Fisons, UK).

Selective insecticide interactions with partial plant resistance

Topical toxicity of pirimicarb. Two concentrations of pirimicarb (Rapid®, Zeneca, UK) (25 and 75 µg ai ml^{-1}) equating, respectively, to *c.* 50 % and 17% of the recommended field dose were applied topically (0.2 µl uncalibrated volume, Arnold Microapplicator Type LV. 65, Burkard, Rickmansworth, UK) to the dorsal surface of second instar nymphs of a laboratory-based clone of *M. persicae* (MpSIL). The insecticide was diluted in AnalaR® grade acetone, and acetone alone was used for the controls. Treated aphids (and controls) were transferred to one of two experimental regimes: a Petri dish experiment or a clip cage

experiment. The former used 3 cm diameter ventilated Petri dishes containing similar diameter leaf discs and dampened filter paper. The Petri dishes were modified to allow escape of second instar nymphs which were subsequently trapped in water-filled 9 cm diameter Petri dish bases which also retained the smaller Petri dishes. Leaf discs were cut from central portions (left or right of midrib) of middle leaves of outdoor-grown, potted, four-week-old *B. oleracea* var. *capitata*, either cvs Derby Day or Minicole. The clip cage experiment involved the use of 18 mm ID nylon mesh-ventilated clip cages which were fixed to the centre-left or -right of the adaxial surface of a middle leaf of individual, potted plants similar to those used in the Petri dish experiment. Five aphids were introduced to each Petri dish or clip cage and each experiment was replicated six times. Mortality within Petri dishes, water baths and clip cages was assessed after 48 h.

Systemic and translaminar activity of a neem seed kernel extract. A neem seed kernel extract (NeemAzal-T/S, Trifolio-M, Lanhau, Germany) was applied at the recommended field concentration (300 µg azadirachtin-A ml^{-1}) as a soil drench (in such a way as to replace the soil water with the neem solution) to two cultivars of potted, outdoor-grown *B. oleracea* var. *capitata* (plant age and growing conditions as above). Distilled water was used in controls. Five second instar nymphs of each of two laboratory-based clones of two aphid species (*M. persicae* [MpSIL] and *B. brassicae* [BbHRI]) were clip-caged to middle leaves (as with pirimicarb experiment, above) of each of the two host plant cultivars (cvs Derby Day and Minicole). The plant pot and soil around the plant stem were covered in aluminium foil to minimise any vapour effects on the aphids. Each treatment was replicated seven times. Systemic activity was also tested by placing the petioles of excised leaves through aluminium foil-covered 100 ml glass beakers containing the neem solution or water controls.

Translaminar activity was tested by applying droplets (50 µl total volume, using a Microman M250® microapplicator, Gilson, France) of neem solution (same concentration) evenly within a 2.5 cm diameter area on the opposite side of the leaf to which a clip cage containing five second instar nymphs had been attached. Two clip-cages of each aphid species were attached to each plant and four plants of each of the two cultivars. Water was used for the controls and each treatment was replicated four times. Mortality and net reproduction of aphids was assessed after 96 h.

Tritrophic interactions with two species of crucifer aphid and a predator

Twelve fourth instar nymphs of each of two laboratory-based clones of *M. persicae* (MpHRI) and *B. brevicoryne* (BbHRI) were transferred to form a cluster in the centre of the abaxial surface of a single middle leaf of individual, four-week-old, outdoor-grown, potted *B. oleracea* var. *capitata* cvs Derby Day and Minicole. Three two-day-old eggs of *A. aphidimyza* (expected to hatch within 24 h) were placed adjacent to each aphid cluster. After 72 h, the number of successfully hatched *A. aphidimyza* larvae were counted, their length measured (using a microscope graticule at x 12 magnification) and the number of adult aphids and offspring recorded. After 6 days, the number of adult and nymphal hosts were counted again. Micropore-ventilated plastic bags (Cryovac®, W.R. Grace, St Neots, UK: 460 x 305 mm, with 15 µm pore size) were placed over each plant and secured around the pot to allow larvae preparing for pupation to burrow in the potting compost and, following pupation, emerge for subsequent scoring. Each treatment was replicated 12 times.

Field studies

Aphid development on several brassica species/ cultivars (Cameron Highlands, Malaysia). Aphid frequency on individual plants and the severity of infestation according to a three level arbitrary scale (light, moderate, heavy) was determined in a field study on the effects on crucifer pests of intercropping different *B. oleracea* var. *capitata* cultivars with *B. pekinensis* on two experimental plots (randomised block designs) on crucifer farms in Boh Road and Mensum Valley (Cameron Highlands, Malaysia) respectively. Data on a range of crucifer pest - natural enemy interactions were collected from the sites, particularly relating to *Plutella xylostella* L. (Lepidoptera: Yponomeutidae). No data relating directly to aphid natural enemies were collected, although an index of relative intensity of aphid infestation was developed to determine any host plant-mediated differences in population development. Owing to site availability, the trials could not be run concurrently (the Boh Road study preceded that of Mensum Valley by about seven weeks, see below). The index was calculated as the product of the proportion of each group of five plants sampled and an arbitrary intensity index (0.5 = light infestation; 1 = moderate infestation; 2 = heavy infestation). The sites were principally used for intercropping studies (with *B. pekinensis* and various cultivars of *B. oleracea* var. *capitata*) so the data used for analysis were derived only from "control" beds of single host plant/cultivars which had not been intercropped. Individual plants were sampled randomly in beds (two-row beds containing approximately forty plants spaced at approximately 40 cm) of each host plant/cultivar; at least 12 plants (from four different beds) of each cultivar were sampled each week over a six week period in both sites (19.07.95–28.08.95 and 11.09.95 –19.10.95 for the Boh Road and Mensum Valley sites respectively). Sampling started three weeks after transplanting and continued until harvest in both sites. Cultivation occurred according to local farmer practice.

Results

Selective insecticide interactions with partial plant resistance

Topical toxicity of pirimicarb. In the Petri dish experiment, mortality of the *M. persicae* clone within Petri dishes increased significantly ($p < 0.05$) between controls and the two pirimicarb concentrations, but although mortality on Minicole was greater than on Derby Day with both concentrations (25 and 75 µg ai ml^{-1}), these differences were not statistically significant ($p > 0.05$) *(Table 1)*. The insecticide or plant cultivar contributed to "restlessness" and resultant escape of aphids from the modified Petri dishes; in three out of four insecticide treatments mortality in the water traps was significantly ($p < 0.05$) greater than for the control with the same cultivar *(Table 1)*. With the highest pirimicarb concentration, the difference in mortalities in water traps between the two cultivars was highly significant ($p < 0.001$) *(Table 1)*. In the clip cage experiment, although Minicole again contributed to greater mortalities in the treatments compared with Derby Day (despite there being zero control mortality with both cultivars), only at the lower concentration was this difference significant ($p < 0.001$) *(Table 1)*.

Systemic and translaminar activity of a neem seed kernel extract. When NeemAzal-T/S was applied at the recommended field concentration (as a soil drench to potted plants of cvs Derby Day and Minicole, mortality of the clones of both aphid species, owing to variance between replicates, was not significantly

Table 1. Toxicity of two concentrations of topically-applied pirimicarb to second instar nymphs of an insecticide-susceptible clone of *Myzus persicae* (MpSIL) under two different experimental regimes (Petri dish and clip cage experiments) and maintained on two different *Brassica oleracea* var. *capitata* cultivars

Pirimicarb concentration (µg ml^{-1})		Mean proportion mortality[a]					
	Cultivar	Total	p^b	Within Petri dishes	p^b	Within water traps	p^b
Petri dish experiment							
0	Derby Day	0 a	NS	0 a	NS	0 a	NS
0	Minicole	0.03 ± 0.01 a		0 a		0.03 ± 0.01 a	
25	Derby Day	0.57 ± 0.09 b	NS	0.43 ± 0.08 b	NS	0.13 ± 0.07 bc	NS
25	Minicole	0.70 ± 0.10 bc		0.50 ± 0.12 b		0.20 ± 0.05 c	
75	Derby Day	0.87 ± 0.08 c	NS	0.83 ± 0.08 c	NS	0.03 ± 0.01 a	***
75	Minicole	0.87 ± 0.13 c		0.77 ± 0.08 c		0.10 ± 0.04 bc	
Clip cage experiment							
0	Derby Day	0 a	NS	–		–	
0	Minicole	0 a		–		–	
25	Derby Day	0.33 ± 0.04 b	***	–		–	
25	Minicole	0.77 ± 0.09 c		–		–	
75	Derby Day	0.87 ± 0.04 cd	NS	–		–	
75	Minicole	0.97 ± 0.03 d		–		–	

[a]Mean proportions ± SE; values within columns followed by a common letter are not significantly ($p > 0.05$) different (5 insects / replicate; 6 replicates / treatment; n = 30) based on ANOVA and pairwise LSD tests on arcsine transformed data.
[b]Significance between cultivars in comparable treatments (LSD tests: NS = not significant; * = $p < 0.05$; ** = $p < 0.01$;*** = $p < 0.001$).

Table 2. Laboratory toxicity of NeemAzal-T/S applied as a soil drench to two cultivars of potted *Brassica oleracea* var. *capitata* with clip-caged second instar nymphs of insecticide-susceptible clones of either *Myzus persicae* or *Brevicoryne brassicae*

Aphid species (clone) Treatment / cultivar		Mortality (mean proportion ± SE)[a]	No. offspring per aphid[a,b]
***Myzus persicae* (MpSIL)**			
Control	Derby Day	0 a	2.60 ± 0.46 a
	Minicole	0 a	2.30 ± 0.70 a
Treatment	Derby Day	0 a	2.00 ± 0.25 ab
	Minicole	0.09 ± 0.06 a	1.66 ± 0.49 abc
***Brevicoryne brassicae* (BbHRI)**			
Control	Derby Day	0 a	1.33 ± 0.29 bcd
	Minicole	0.07 ± 0.04 a	0.65 ± 0.04 d
Treatment	Derby Day	0.12 ± 0.05 a	0.80 ± 0.11 cd
	Minicole	0.04 ± 0.03 a	0.93 ± 0.33 cd

[a]Values within columns followed by a common letter are not significantly ($p > 0.05$) different (4 replicate plants; 2 clip-cages/plant; 5 aphids/clip-cage; n = 40) based on ANOVA and pairwise LSD tests; proportion data arcsine transformed prior to analysis.
[b]Experimental insects at N2 stage on Day 0 and reproduction assessed after 4 days.

Table 3. Tritrophic bionomics of a three cultivar (*Brassica oleracea* var. *capitata*) - two aphid clone (*Myzus persicae*: MpHRI; *Brevicoryne brassicae*: BbHRI) - predator (*Aphidoletes aphidimyza*) laboratory-based system

	3 days				6 days			
Aphid clone Cultivar	Predator recovery (mean proportion ± SE)	Predator body length (mm)	No. live hosts per replicate[a]		No. live hosts per replicate[a]		Emergence rate[b] (mean proportion ± SE)	Predator survival to adult[c] (mean proportion ± SE)
			Adults	Nymphs	Adults	Nymphs		
MpHRI								
Derby Day	0.25±0.09ab	2.19±0.14a	6.67±0.40ac	16.83±2.89a	6.00±0.76a	21.75±3.71ab	0.81±0.12a	0.17±0.05ab
Minicole	0.22±0.08a	1.54±0.19bc	8.75±0.51b	17.50±2.39a	5.58±0.83a	20.5±3.05ab	0 c	0 c
Ruby Ball	0.25±0.05a	1.11±0.15d	7.67±0.63ab	16.25±2.70a	3.75±0.60ab	24.08±3.22a	0.46±0.16ab	0.06±0.04c
BbHRI								
Derby Day	0.44±0.11b	1.84±0.06b	5.25±0.54cd	7.83±1.38b	4.75±1.01ab	15.00±3.80bc	0.74±0.21a	0.22±0.06a
Minicole	0.17±0.06a	1.61±0.12bc	4.67±0.61d	6.00±1.40b	2.83±0.61b	5.00±1.77d	0.17±0.17bc	0.03±0.03c
Ruby Ball	0.11±0.03a	1.52±0.04c	5.58±0.53cd	7.58±1.40b	4.08±0.92ab	11.33±2.26cd	0.50±0.29ab	0.08±0.04bc

Within columns, means followed by a common letter are not significantly (p >0.05) different (ANOVA and pairwise LSD tests). Proportion data arcsine transformed prior to analysis.
[a]Twelve late stage nymphs and three *A. aphidimyza* eggs introduced to each replicate at Day 0.
[b]Proportion emerging as adults based on number recovered from each treatment on Day 3.
[c]Total survival from egg to adult (36 eggs transferred per treatment; 12 replicates).

(p > 0.05) different compared with the controls *(Table 2)*. In addition, there were no significant (p > 0.05) effects between treatments and controls in terms of number of offspring produced over four days, as well as no significant (p > 0.05) cultivar-mediated effects on number of offspring produced *(Table 2)*. In contrast, in the second systemic test, when petioles of infested excised leaves were placed in solutions of NeemAzal-T/S, mortality with both species was 100% (zero control mortality). Complete mortality of clones of both species was also caused by the neem extract in the translaminar tests (also with zero control mortality).

Tritrophic interactions with two species of crucifer aphid and a predator

Within species, there were no significant (p > 0.05) differences in the number of *A. aphidimyza* larvae recovered between the three cultivars, except with the *B. brevicoryne* clone, where recovery on Derby Day was greater than double (44 %*;* p < 0.05) that on the other two cultivars *(Table 3)*. In the case of both aphid clones, there was a consistent trend for predator larval body length (on Day 3) to be smaller through the cultivar series Derby Day > Minicole > Ruby Ball, although these differences were not significant (p >0.05) in one comparison (with *B. brevicoryne*, between Derby Day and Minicole) *(Table 3)*. Nevertheless, body length was consistently greater (p <0.05) on Derby Day compared with the other two cultivars with both aphid species as hosts. No clear, statistically significant patterns emerged with respect to number of live adult or nymphal hosts available to predators, except that nymph availability was significantly (p <0.05) less on Minicole and Ruby Ball on both Days 3 and 6 with *B. brevicoryne*, compared with *M. persicae (Table 3)*. However, when adult emergence of the recovered (Day 3) *A. aphidimyza* was compared between cultivars and aphid clones, Minicole was responsible for substantial (p <0.05) mortality with both clones: in the case of the *M. persicae* clone, emergence was zero (compared with 81% on Derby Day and 46% on Ruby Ball) and slightly higher (17%) for the *B. brevicoryne* clone (compared with 74% and 50% on Derby Day and Ruby Ball, respectively) *(Table 3)*. Only about one in five predators survived from egg to adult on Derby Day with both host clones (no significant [p >0.05] differences), but these survival rates were nearly three-fold greater (p < 0.05) than survival on Ruby Ball. Again, for both clones, net survival was greater on Derby Day (but not significantly [p <0.05]) than survival on Minicole, with no significant (p >0.05) differences between the clones *(Table 3)*.

Field studies

Aphid development on several brassica species/ cultivars (Cameron Highlands, Malaysia). When relative intensity of *M. persicae* infestation on three host plant/cultivars was compared between two sites (the data from weekly assessments being pooled over the majority of the cropping period during which the studies were undertaken), substantial differences in *M. persicae* infestation were noted, with no consistent trends between host plant/cultivars *(Figure 1)*. The two most heavily aphid-infested plant groups were Scarlet O'Hara (*B. oleracea* var. *capitata*; red-leaved) in Mensum Valley and Super Queen (*B. pekinensis*) in Boh Road *(Figure 1)*.

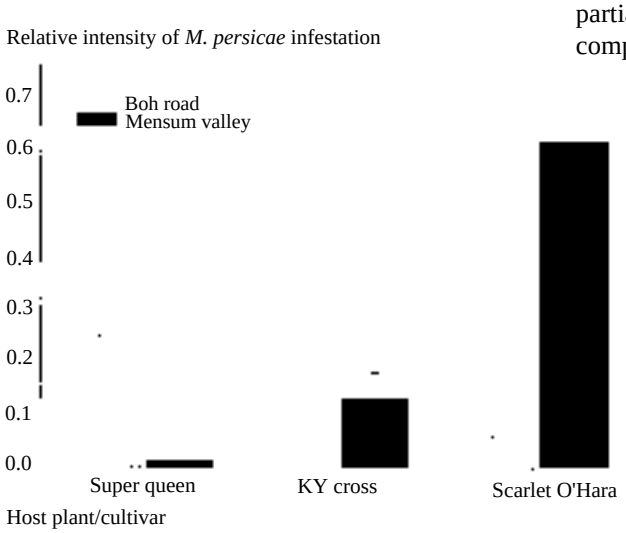
Discussion

The pirimicarb experiments, employing substantially less than recommended rates of pirimicarb, demonstrated significant (two- to three-fold) differences in insecticide toxicity to *M. persicae* between the two cultivars. The consistently greater mortality within water traps with Minicole compared with Derby Day (although not significant with the control and lower concentration of pirimicarb) may have been caused, in part, by a degree of antixenosis. Additive or synergistic effects between such antixenosis and the higher insecticide concentration appeared a likely cause of the significantly greater rate of escapism into the water traps with Minicole (cf. Derby Day). Such a mechanism, causing "restlessness" in *M. persicae*, could potentially be used to advantage in the field. The resultant increased mobility and chance of aphids falling off plants would be likely to increase the predation rate of aphids and the selectivity of pirimicarb would minimise the likelihood of secondary toxicity to natural enemies. Further experimentation with other insecticides and different sources of plant resistance, as well as field testing, are necessary to better evaluate the field potential of such interactions.

The clip cage experiment with pirimicarb also revealed an interaction which has relevance to the field: insecticide topically applied to nymphs of *M. persicae* maintained on the partially resistant Minicole (whole plants), suffered statistically equivalent mortality compared with nymphs treated with a three-fold greater insecticide concentration then maintained on the susceptible cultivar, Derby Day (77% and 87% respectively). This supports the notion of a judicious insecticide use - partial plant resistance - biological control strategy, advocated by many workers in integrated pest management (IPM) (e.g., Stern *et al.*, 1959; van Emden, 1989).

In theory, a selective insecticide with true systemic properties could provide an important means of providing high levels of control of sucking pests while minimising insecticide exposure of natural enemies searching the foliar environment. For this reason, the neem extract, NeemAzal-T/S, was investigated. However, the systemic ability of the product appeared to be rather limited, causing measurable but not significant mortality to both *M. persicae* and *B. brevicoryne* when applied as a soil drench to potted plants in the laboratory. In contrast with the pirimicarb studies, cultivar-mediated differences in mortality were not found. However, NeemAzal-T/S did cause 100% mortality of both aphid species when petioles of excised leaves were placed in a solution at the recommended field concentration, as well as when it was tested for translaminar activity. Such a product, with a known degree of selectivity (Schmutterer, 1990) may be an important candidate insecticide in IPM programmes for crucifer aphids, where accurate targeting of the pest with insecticides, insecticide resistance to carbamates, organophosphates and pyrethroids, as well as resurgence have been widespread problems.

The tritrophic experiment with two partially resistant and one susceptible cabbage cultivar, both aphid species and the predatory gall midge, *A. aphidimyza*, demonstrated a sensitivity of the predator to factors related to the partially resistant cultivars. This was noted, early in the experiment (Day 3), with shorter body lengths in the predatory larvae on the partially resistant cultivars (Minicole and Ruby Ball) compared with the susceptible cultivar (Derby Day).



[a]Based on a weighted relative index determined as the product of the proportion of each group of 5 plants sampled and an intensity factor (0.5 = light infestation; 1 = moderate infestation; 2 = heavy infestation) over six weeks at weekly intervals (minimum 12 plants sampled /week / cv). Error bars represent standard errors.
[b]*Brassica pekinensis* cv Super Queen; *B. oleracea* var. *capitata* cvs KY Cross (green) and Scarlet O'Hara (red).
[c]Southern zone, Cameron Highlands; approx. 1000 m altitude.
[d]Central zone, Cameron Highlands; approx. 1500 m altitude.

Figure 1. Relative intensity of infestation[a] by Myzus persicae on three plant groups[b] over six weeks of a cropping period on two sites (Boh Road[c] and Mensum Valley[d]) in the Cameron Highlands, Malaysia (August - September 1995)

The effect on adult predator emergence was most dramatic, particularly on Minicole, with negligible emergence from predators with *B. brevicoryne* as hosts, and no emergence at all in the comparable *M. persicae* experiment. These laboratory results indicate a potential incompatibility between partial plant resistance and predatory control of crucifer aphids with *A. aphidimyza*, and was probably mediated by antibiosis factors (secondary plant chemicals) in the partially resistant plants. Similar incompatibilities have been found and discussed by other authors (e.g., van Emden, 1991; Hare, 1992). The nature of the interaction will be subsequently studied with other predator groups, such as the Syrphidae, Anthocoridae and Coccinelidae, which tend to be more important biological control agents in the field. Different tritrophic interactions may well occur with parasitoids in crucifer aphid-based systems, and these will also be investigated.

The field studies from two adjacent zones of the Cameron Highlands (Malaysia) emphasised the considerable degree of variation in infestation of *M. persicae* which may result on different crucifer host plants/cultivars between nearby upland regions. Such variation is the product of a wide range of interacting factors including aphid clone-host plant adaptation, physiological variation in host plants, differing degrees of tolerance or resistance to insecticides, varying activity of natural enemies and climatological/altitude differences. In order to better appreciate the processes that mediate this variation, it is important to study controlled factors in the laboratory alongside more detailed field studies which measure both abiotic and biotic parameters relating to three or four trophic levels.

These preliminary studies highlight some of the complexities inherent in IPM systems. They demonstrate particular interactions between insecticides and partial host plant resistance which may be able to be exploited in control programmes. However, they also point to a possible incompatibility between partial plant resistance and one predatory species. Such tritrophic studies, with and without selective insecticides, are likely to provide an important means of finding optimum balances between the many tactics employed in IPM of crucifer pests.

Acknowledgements

This research was supported by the Leverhulme Trust and we thank MARDI for cooperating with our field research on crucifer pests in the Cameron Highlands between 1994 and 1996.

References

Hare, D. J. (1992). Effects of plant variation on herbivore-enemy interactions. In: *Plant Resistance to Herbivores and Pathogens - Ecology, Evolution and Genetics*. Fritz, R. S. and Simms, E. L. (Eds.) Chicago & London: University of Chicago Press, p. 278–298.

Herzog, D. C. and Funderbunk, J. E. (1985). Plant resistance and cultural practice interaction with biological control. In: *Biological control in Agricultural IPM Systems*. Hoy, M. A. and Herzog, D. C. (Eds.) New York: Academic Press, Inc., p. 67–88.

Price, P. W., Westoby, M., Rice, B., Atsatt, P. R., Fritz, R. S., Thompson, J. M. and Mobley, K. (1986). Parasite mediation in ecological interactions. *Annual Review of Ecology and Systemmatics* **17**: 487–505.

Schmutterer, H. (1990). Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. *Annual Review of Entomology* **35**: 271–297.

Stern, V. M., Smith, R .F., van Den Bosch, R. and Hagen, K.S. (1959). The integration of chemical and biological control of the spotted alfalfa aphid. 1. The Integrated Control Concept. *Hilgardia* **29**: 81–101.

van Emden, H. F. (1989). *Pest Control*. London: Edward Arnold. 117 pp.

van Emden, H. F. (1991). The role of host plant resistance in insect pest mis-management. *Bulletin of Entomological Research* **81**:123–126, 1991.

Verkerk, R. H. J. and Wright, D. J. (1996). Multitrophic interactions and management of the diamondback moth. *Bulletin of Entomological Research* **86**: 205–216.

Insecticide resistance in diamondback moth, *Plutella xylostella* (L.), in southern Australia

Nancy M. Endersby and Peter M. Ridland

Agriculture Victoria, Institute for Horticultural Development, Private Bag 15, South Eastern Mail Centre VIC 3176, Australia

Abstract

Synthetic pyrethroids have been the most widely used insecticide group for control of *Plutella xylostella* in southern Australia since the early 1980's. Insecticide resistance in Australian populations of *P. xylostella* was first confirmed in Queensland in 1987. Since then, resistant populations have developed throughout southern Australia with reports of insecticide control failures coming from South Australia (1990), Victoria (1993), New South Wales (1993), Tasmania (1995) and Western Australia (1995).

Leaf dip bioassays were conducted to determine the toxicity of permethrin to 21 populations of *P. xylostella* collected from brassica crops grown in Victoria, Western Australia, South Australia, Tasmania and Queensland. High levels of resistance to permethrin (13 to 62 times greater than susceptible laboratory-reared populations) were found in populations collected from brassica crops where growers had experienced difficulties in controlling *P. xylostella* with synthetic pyrethroids.

Preliminary testing with methamidophos suggests that resistance levels of *P. xylostella* populations in southern Australia to this insecticide are not as high as those to permethrin. Low levels of resistance (two to 12.5 times greater than susceptible laboratory-reared populations) were recorded from three field populations.

Key words: insecticide resistance, crucifers, brassica, diamondback moth, *Plutella xylostella*, synthetic pyrethroids

Introduction

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), has become the most destructive insect pest of brassica crops throughout the world (Talekar and Shelton, 1993). Over the last 20 years it has caused increasing problems due to the development of resistance to many classes of insecticide, particularly throughout Asia (Cheng, 1988) and the US (Shelton *et al*., 1993). In Australia, resistance to a range of insecticides was first confirmed in Queensland where control failures were first experienced about ten years ago (Anon., 1987) and in South Australia where control failures were reported in 1990 (G. Baker, pers. comm.). Resistance levels in *P. xylostella* in these states were estimated using topical application bioassays. Failures with synthetic pyrethroids were first reported by Victorian growers in autumn 1993. In the summer/autumn period of 1993/94, control failures were widespread throughout all brassica production areas of the state. Control problems also occurred in NSW during a similar time period (V. Rajakulendran, pers. comm.). Insecticide failures against *P. xylostella* were reported for the first time by growers in Western Australia and Tasmania in autumn 1995.

This paper reports a study to document resistance levels to the widely used synthetic pyrethroid, permethrin, in populations of *P. xylostella* from southern Australia. Some results from bioassays with the organophosphate, methamidophos, are also presented. The survey is the first use in Australia of the leaf dip bioassay for estimation of resistance levels in *P. xylostella*.

Materials and Methods

In 1994, eight populations of *P. xylostella* were collected from the main brassica growing areas of Victoria: Werribee South (37º 58' S 144º 41' E), Cranbourne (38º 07' S 145º 17' E), Dandenong (38º 02' S 145º 13' E), Lysterfield (37º 54' S 145º 18' E), Lang Lang (38º 16' S 145º 34' E), Lindenow (37º 48' S 147º 28' E), Boisdale (37º 53' S 146º 59' E) and Mildura (34º 11' S 142º 09' E). An additional population was collected from a non-commercial trial plot at Knoxfield (37º 53' S 145º 15' E).

In 1995, two populations were collected from South Australia: Koppamurra (37° 04' S 140° 48' E) and Naracoorte (36º 58' S 140º 45' E) and Western Australia: Perth (31º 57' S 115º 51' E) and Manjimup (34º 15' S 116º 09' E). Three populations were collected from Tasmania: Wesleyvale (41º 12' S 146º 27' E), Devonport (41º 11' S 146º 21' E) and Forth (41° 12' S 146° 15' E). Four populations were collected from Victoria: Geelong (38º 10' S 144º 21' E), Myrtleford (36º 34' S 146º 44' E), Yarra Junction (37º 47' S 145º 37' E) and Keysborough (38° 00' S 145° 10' E).

In 1996, one population was collected from Queensland: Tenthill (27° 34' S 152° 15' E). The locations of all populations are shown in *Figure 1*. Apart from the Knoxfield, Lang Lang, Devonport and Forth populations, *P. xylostella* populations were

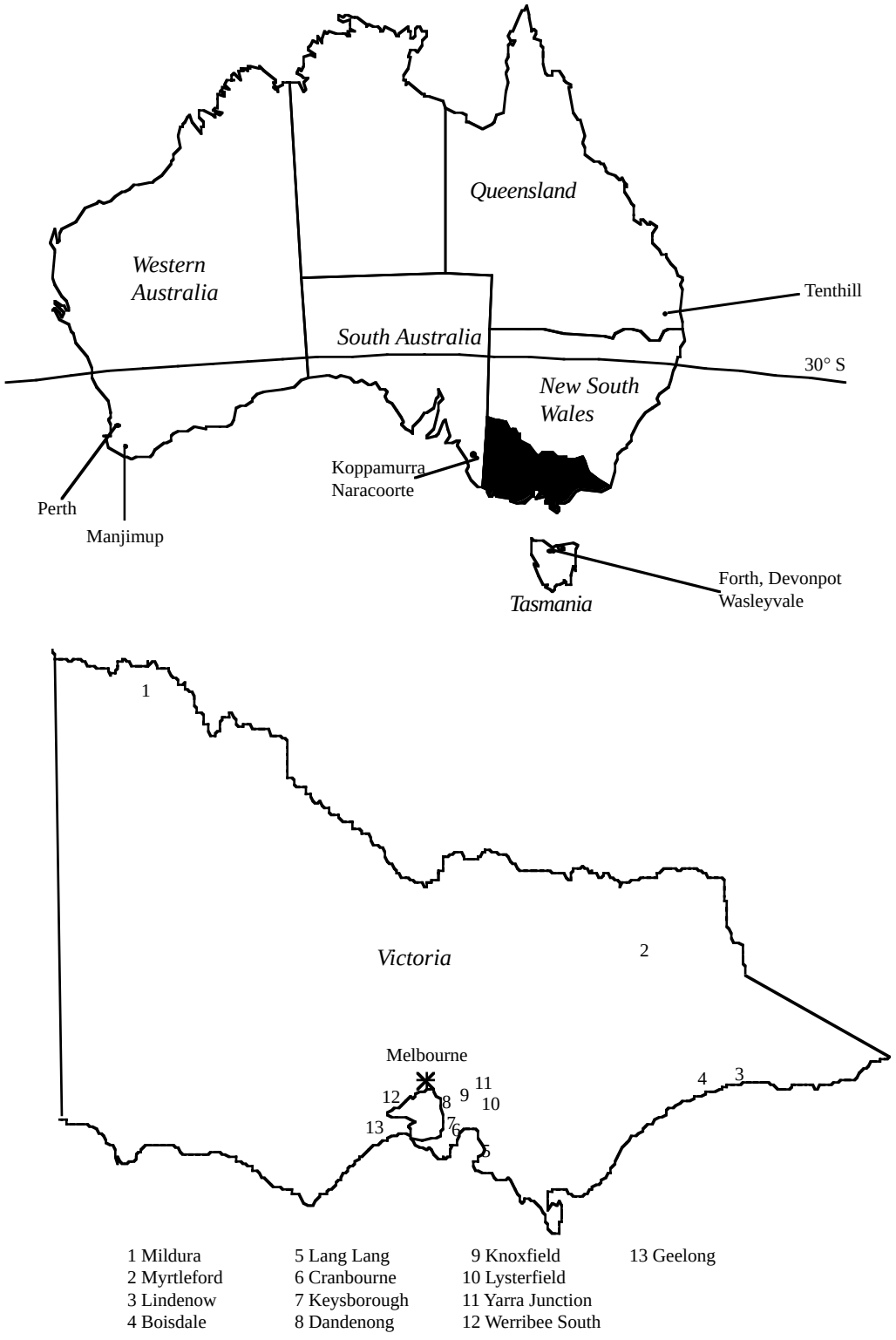


Figure 1. Location of Plutella xylostella populations tested for insecticide resistance in Australia with leaf dip bioassay

collected from properties where insecticide control failures had occurred.

Field collected *P. xylostella* larvae were reared on cabbage seedling leaves in the laboratory at 25ºC (16L : 8D photoperiod). Susceptible laboratory populations of *P. xylostella* were obtained from the University of Adelaide, Department of Crop Protection, Waite Campus, SA and the Biological and Chemical Research Institute, Rydalmere, NSW.

A leaf dip bioassay after Tabashnik and Cushing (1987) was adopted. Cabbage leaf discs of 6 cm diameter were dipped for 5 s in distilled water solutions of formulated permethrin (500 g/L Ambush®, ICI) or methamidophos (580 g/L Nitofol®, Bayer) and hung vertically to dry in a fume hood for 2 h. Control discs were dipped in distilled water only. No wetting agents were used. Discs were placed in 8 cm diameter Petri dishes. Eight to twelve third instar *P. xylostella* were added to each disc and allowed to feed at 28 ºC. Mortality was assessed at 48 h. Dead larvae were scored as those which did not move when touched with a paintbrush. Probit analysis (POLO-PC, LeOra

Software) was used to estimate LC_{50} and slope for each population.

Results and Discussion

Results of the survey of *P. xylostella* from southern Australia show that high levels of resistance to permethrin are present in field collected populations from areas where insecticide control failures have occurred. Among the thirteen Victorian field collected populations of *P. xylostella*, the populations from Mildura, Cranbourne and Geelong showed highest levels of resistance to permethrin *(Figure 2; Tables 1, 2)*. *P. xylostella* from Keysborough were susceptible to permethrin. Populations from Lang Lang and Yarra Junction showed resistance levels intermediate to those of the laboratory populations (Waite and Rydalmere) and those of the other field collected populations. Resistance levels of the other field collected populations ranged from 13 to 43 times the level of the laboratory populations.

P. xylostella from the Waite and Rydalmere laboratory populations proved very susceptible to permethrin and showed a similar response. We now routinely use a diagnostic dose of 0.1 mg/ml to detect resistance to permethrin in field populations.

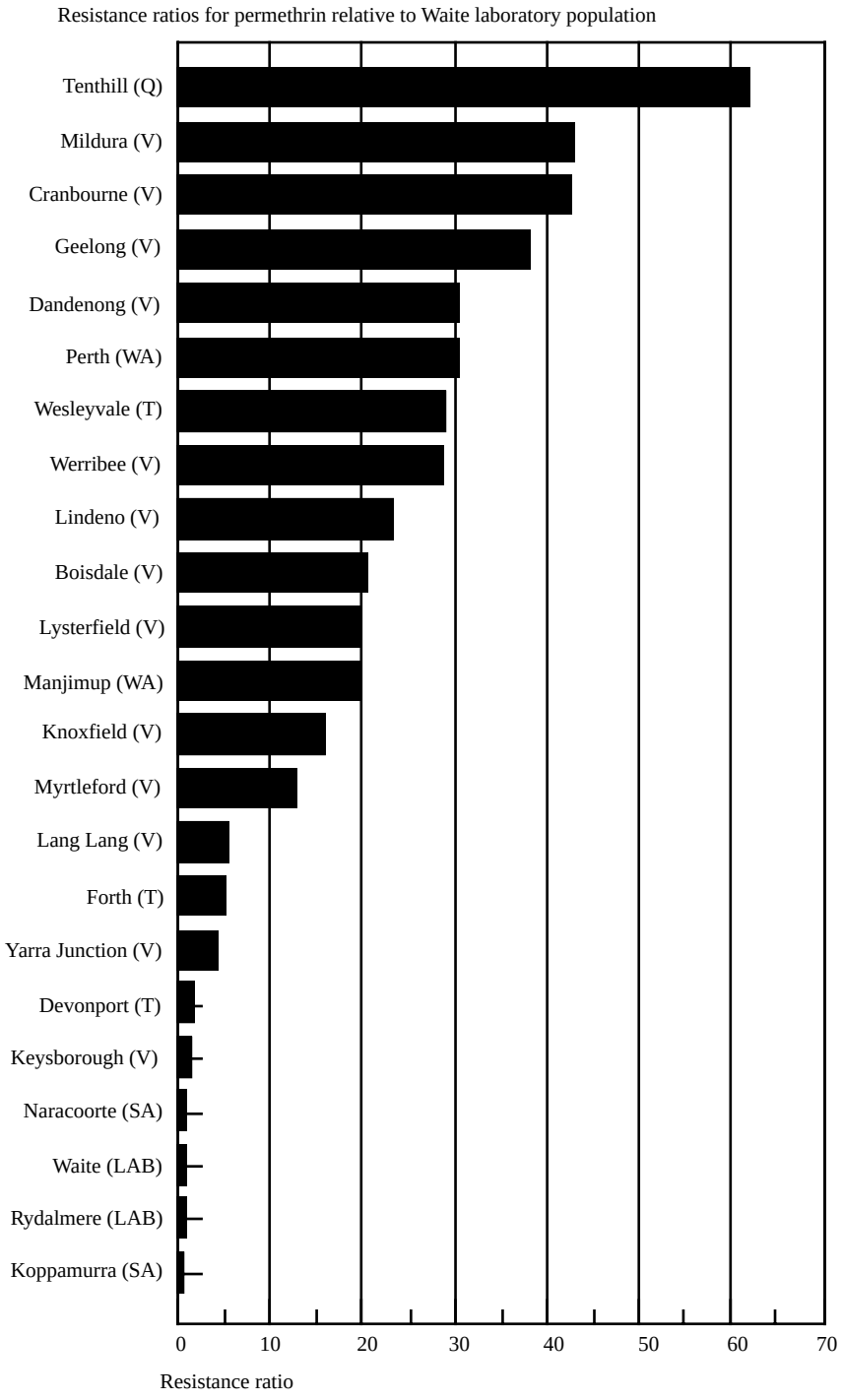


Figure 2. Resistance ratios for permethrin for 21 Australian Plutella xylostella populations, determined by dividing the LC_{50} for a population by the LC_{50} determined for the Waite laboratory population in the year tested

Table 1. Concentration-mortality responses of nine Victorian populations of *Plutella xylostella* (1994) and two laboratory populations (Rydalmere and Waite) to permethrin

Population	Gen[a]	n[b]	Slope ± s.e.	LC_{50} mg a.i./ml (95% CL)	RR[c]
Mildura	F4	435	3.67 ± 0.39	**0.260** (0.302 - 0.218)	43.3
Cranbourne	F4	192	2.61 ± 0.57	**0.257** (0.408 - 0.132)	42.8
Dandenong	F3	284	1.57 ± 0.23	**0.184** (0.258 - 0.130)	30.7
Werribee	F1	268	2.58 ± 0.31	**0.173** (0.232 - 0.132)	28.8
Lindenow	F1	276	2.39 ± 0.35	**0.140** (0.194 - 0.094)	23.3
Boisdale	F3	160	2.74 ± 0.38	**0.121** (0.177 - 0.080)	20.7
Lysterfield	F1	277	1.38 ± 0.16	**0.120** (0.190 - 0.079)	20.0
Knoxfield	F7	182	1.75 ± 0.30	**0.096** (0.188 - 0.062)	16.0
Lang Lang	F3	287	1.47 ± 0.18	**0.034** (0.046 - 0.024)	5.67
Waite	-	270	3.11 ± 0.52	**0.006** (0.008 - 0.002)	1.00
Rydalmere	-	280	2.08 ± 0.36	**0.005** (0.007 - 0.003)	0.83

[a] Number of generations reared in laboratory before testing, **[b]** Number of subjects, **[c]** RR is the resistance ratio determined by dividing the LC_{50} for a population by the LC_{50} for the Waite population

Table 2. Concentration-mortality responses of ten populations of *Plutella xylostella* from southern Australia (1995) and one laboratory population (Waite) to permethrin

State	Population	Gen[a]	n[b]	Slope ± s.e	LC_{50} mg a.i./ml (95% CL)	RR[c]
Victoria	Geelong	F4	360	1.66 ± 0.20	**0.498** (0.313 - 0.781)	38.3
	Myrtleford	F4	278	1.55 0.16	**0.168** (0.121 - 0.231)	12.9
	Yarra Junction	F4	285	1.87 0.23	**0.057** (0.036 - 0.081)	4.39
	Keysborough	F4	270	1.22 0.17	**0.021** (0.009 - 0.034)	1.62
Tasmania	Wesleyvale	F2	354	2.03 0.19	**0.379** (0.218 - 0.801)	29.2
	Devonport	F2	279	1.97 0.21	**0.023** (0.017 - 0.030)	1.77
Western Australia	Perth	F2	362	2.19 0.27	**0.398** (0.294 - 0.528)	30.6
	Manjimup	F3	275	2.40 0.28	**0.255** (0.191 - 0.336)	19.6
South Australia	Naracoorte	F1	267	1.44 0.20	**0.016** (0.006 - 0.027)	1.23
	Koppamurra	F1	275	1.70 0.30	**0.009** (0.004 - 0.016)	0.69
Laboratory Population	Waite	-	281	2.60 0.26	**0.013** (0.010 - 0.017)	1.00

[a] Number of generations reared in laboratory before testing, **[b]** Number of subjects, **[c]** RR is the resistance ratio determined by dividing the LC_{50} for a population by the LC_{50} for the Waite population

Table 3. Concentration-mortality responses of populations of *Plutella xylostella* from Queensland and Tasmania and a laboratory population (Waite) (1996) to permethrin

State	Population	Gen[a]	n[b]	Slope ± s.e	LC_{50} mg a.i./ml (95% CL)	RR[c]
Queensland	Tenthill	F1	336	1.77 0.25	**0.811** (0.559 - 1.120)	62.4
Tasmania	Forth	F9	197	1.79 0.21	**0.071** (0.049 - 0.101)	5.46
Laboratory Population	Waite	-	160	1.92 0.25	**0.013** (0.009 - 0.018)	1.00

[a] Number of generations reared in laboratory before testing, **[b]** Number of subjects, **[c]** RR is the resistance ratio determined by dividing the LC_{50} for a population by the LC_{50} for the Waite population

Both Western Australian *P. xylostella* populations tested were resistant to permethrin, with the Perth population having a higher resistance ratio (RR = 30.6) than the population from Manjimup (RR = 19.6). Both populations tested from South Australia in 1995 were susceptible to permethrin, suggesting that the control failures were due to factors other than resistance to permethrin.

Of the populations tested from Tasmania in 1995 *(Table 2)*, one was resistant (Wesleyvale) and one was susceptible to permethrin (Devonport). The Forth population from Tasmania had a resistance ratio of 5.46 *(Table 3)*, intermediate between Wesleyvale and Devonport. The population of *P. xylostella* from Tenthill, Queensland was found to have the highest

Table 4. Concentration-mortality responses of three populations of *Plutella xylostella* from southern Australia and a laboratory population (Waite) to methamidophos

State	Population	Gen[a]	n[b]	Slope ± s.e	LC_{50} mg a.i./ml (95% CL)	RR[c]
1994						
VICTORIA	Lysterfield	F2	281	1.87 ± 0.20	**0.181** (0.134 - 0.258)	3.35
LABORATORY POPULATION	Waite	-	291	2.05 ± 0.22	**0.054** (0.036 - 0.075)	1.00
1995						
WESTERN	Perth	F2	338	3.66 ± 0.64	**0.825** (0.670 - 1.008)	12.5
AUSTRALIA	Manjimup	F3	296	2.78 ± 0.38	**0.150** (0.113 - 0.185)	2.27
LABORATORY POPULATION	Waite	-	282	3.28 ± 0.56	**0.066** (0.048 - 0.081)	1.00

[a] Number of generations reared in laboratory before testing, [b] Number of subjects, [c] RR is the resistance ratio determined by dividing the LC_{50} for a population by the LC_{50} for the Waite population

resistance ratio (RR = 62.5) of the populations tested in this study *(Table 3)*.

Preliminary testing with methamidophos suggests that resistance levels *(Table 4)* of *P. xylostella* populations in southern Australia to this insecticide are not as high as those to permethrin. Low resistance ratios were recorded for the Lysterfield (Victoria) population (RR = 3.35) and the Manjimup (Western Australia) population (RR = 2.27) *(Table 4)*. The highest ratio was that of the Perth (Western Australia) population (RR = 12.5). Further testing with methamidophos is required.

Although insecticide control failures of *P. xylostella* have been widespread throughout Victoria since 1993, some growers were able to minimise damage by improvement of spray application and timing and use of *Bacillus thuringiensis*. Registration of several new chemical groups in Australia in the near future will allow a resistance management strategy to be deployed involving rotation of chemical groups and *B. thuringiensis* in combination with crop monitoring and cultural practices such as improved hygiene. Future research on *P. xylostella* in Australia is being focussed on development and implementation of a robust resistance management strategy.

Acknowledgments

We thank Dr M. Keller and Dr V. Rajakulendran for supplying the susceptible *P. xylostella* populations.

References

Anonymous (1987). Entomology Branch Annual Report 1986–87. Department of Primary Industries, Queensland Government.

Baker, G. (1990). Personal communication. Entomology Unit. SARDI, Adelaide, Australia.

Cheng, E. Y. (1988). Problems of control of insecticide-resistant *Plutella xylostella*. *Pesticide Science* 23: 177–188.

Rajakulendran, V. (1994). Personal communication. NSW Agriculture, BCRI, Rydalmere, Australia.

Shelton, A. M., Wyman, J. A., Cushing, N. L., Apfelbeck, K., Dennehy, T. J., Mahr, S. E. R. and Eigenbrode, S. D. (1993). Insecticide resistance of diamondback moth (Lepidoptera: Plutellidae) in North America. *Journal of Economic Entomology* 86: 11–19.

Tabashnik, B. E. and Cushing, N. L. (1987). Leaf residue vs. topical bioassays for assessing insecticide resistance in the diamondback moth, *Plutella xylostella* L. *FAO Plant Protection Bulletin* 35: 11–14.

Talekar, N. S. and Shelton, A. M. (1993). Biology, ecology and management of the diamondback moth. *Annual Review of Entomology* 38: 275–301.

Diamondback moth: Feeding preference among commercial varieties of head cabbage

R.S. Shimabuku[1], R.F.L. Mau[2] and L. Gusukuma-Minuto[2]

[1]Hawaii Cooperative Extension Service, College of Tropical Agriculture and Human Resources, University of Hawaii, Kahului, HI 96732

[2]Department of Entomology, University of Hawaii at Manoa, 3050 Maile Way, Rm. 310, Honolulu, HI 96822.

Abstract

Thirty-nine commercial varieties of head cabbage were evaluated for feeding preference of the diamondback moth (DBM), *Plutella xylostella,* at Kula, Hawaii, USA. Varieties were evaluated for density of caterpillars, extent of feeding damage and percentage of marketable heads. Twelve out of 39 (31%) varieties that showed the least DBM caterpillar damage and produced high percentage of marketable heads were: Golden Cross; Heads Up; Pacifica; Tropical Delight; Eastern Riches; Good Seasons; Fast Vantage; Stonehead; Genuine; Gourmet Y.R. 11646S; Scorpio; and Blue Thunder. These varieties were ranked with low caterpillar damage of 1.33 or less (0=no damage, 5=extensive damage) and produced 80% or more marketable heads. Nine of these 12 varieties also had fewer diamondback moth larvae and pupae (0.67) per head, while the other 3 varieties (Tropical Delight, Blue Thunder and Gourmet Y.R. 11646S) had 0.77 to 1.03 larvae and pupae per head.

Key words: feeding preference, IPM, damage rating, DBM, cabbage

Introduction

In Hawaii, USA, approximately 162 hectares of head cabbage are planted each year. The primary growing areas are in Kula, Maui and Kamuela, Hawaii. The major pest of crucifers is the diamondback moth (DBM), *Plutella xylostella* (Linnaeus), (Lepidoptera, Plutellidae).

In the 1980s, the DBM reached epidemic proportions due to a buildup in pesticide resistance to synthetic pyrethroids. Other outbreaks occurred in 1991, 1992 and 1994 during the summer months of April to September in Kula and Kamuela. Cabbage growers experienced yield reductions of 20 to 40%, and in some cases up to a 100%. Presently, growers used an integrated approach for the control of the DBM including chemical and non-chemical methods, *Bacillus thuringiensis* (Bt) strains, and scouting techniques. However, with increasing number of pesticides being lost due to increased environmental and human health concerns and the buildup of pesticide resistance, growers will have to look for alternatives to control DBM. This study examines the feeding preference of the DBM among 39 commercial varieties of head cabbage.

Materials and Methods

A field experiment was conducted during 1995 at the Kula Research Substation, Hawaii, USA. The substation is located on the westward slopes of Mt. Haleakala at an elevation of 466 meters. During the field experiment the average ambient temperature was 20.3 °C with a minimum and maximum temperature of 12.8 °C and 28.9 °C, respectively. The total rainfall during this period was 45.7 cm. The experiment was installed in a randomized complete block design with 39 treatments replicated three times. Each treatment consisted of a 30.5 m row of a cabbage variety spaced 45.7 cm between and in rows. Adjacent rows were staggered to allow equidistant plant spacing. The blocks were separated by a 3.0 m wide space to allow sprayer access. Total field dimensions were 97.5 m by 18.3 m. A preplant application of the herbicide, Oxyflurofen (Goal 1.6) was applied at the rate of 0.54 kg of AI/ha. To simulate grower practices, *B. thuringiensis var aizawai* (XenTari) was applied at 0.56 kg of AI/ha to reduce high populations of DBM to assure that there would be plants at harvest. Fungicide sprays of Dithiocarbamate 1.79 kg of AI/ha were alternated with Chlorothalonil 1.26 kg of AI/ha and applied on a weekly basis to control fungal diseases. The spray adjuvant used was Excel 90 6.56 ml/378.5 liters + Bond 177.42 ml/378.5 liters.

Pre-treatment surveys for caterpillars were made one week after planting. Subsequent field evaluations were made two times during the cropping cycle. These evaluations were conducted during the cupping (4 weeks after transplanting) and harvest stage (8–10 weeks after transplanting). Post-treatment observations consisted of randomly selecting and removing five plants per plot. In the final/harvest survey, 10 plants per plot were randomly selected. In each survey, all the leaves from each plant were carefully examined for larvae. Larvae were identified and data collected on the pest species and relative developmental stages of the larvae.

Cabbage varieties mature at different times and each was harvested at optimum maturity. Harvest data was collected on April 25 and May 8, 1995. At each harvest, ten randomly selected mature heads from each plot were cut and trimmed as though they would be marketed commercially. Heads were individually weighed and assessed for marketability after insect

counts. Marketability ratings were evaluated by severity of insect damage, shape and color of each head. Unmarketable heads had extensive damage to interior leaves. Severity of insect damage (0=no damage, 5=extensive damage) was rated prior to insect counts.

For analysis, only data from the harvest survey were used. Data were analyzed by analysis of variance and Tukey's mean separation test (SAS for Windows version 6.08) was used on percent of marketable heads, qualitative damage scores and mean number of DBM larvae and pupae per plant.

Results and Discussion

The predominant pest encountered was the DBM which made up an estimated 95% of the caterpillars found. The remaining 5% were other lepidopteran pests such as imported cabbage worm, *Pieris rapae* (Linnaeus); imported cabbage webworm, *Hellula undalis* (Fabricius); and the cabbage looper, *Trichoplusia ni* (Hubner). The top 12 varieties produced 80% or more marketable heads. Of these varieties, Heads Up and Golden Cross produced a significantly higher percentage of marketable heads than five of the top 12 varieties *(Table 1)*. Using a ranking scale of 0 to 5 (no damage to extensive damage), the top 12 varieties had low caterpillar damage with ratings of 1.33 or less *(Table 3)*. Blue Thunder and Stonehead, were exceptions with ratings of 1.47 and 2.07, respectively. Ratings of the top 12 varieties ranged from 0.70 to 1.37, but the ratings were not significantly different. Also, the top 12 varieties sustained damage primarily on the outer head and wrapper leaves. The varieties with low percentage of marketable heads, sustained leaf damage far into the head, rendering it unmarketable. The top varieties also had fewer diamondback moth larvae and pupae (0.67) per plant compared to other varieties, with the exception of 'Tropical Delight', 'Blue Thunder' and 'Good Seasons' with 0.77, 0.90 and 1.13, respectively *(Table 2)*. The industry standard, 'Tastie' is a variety preferred by the DBM and is highly susceptible to larval damage. It produced 30% marketable heads, had a damage rating of 2.37 and a mean of 1.67 DBM larvae and pupae per plant. The other industry standard, 'Scorpio'; a non preferred variety by the DBM, produced 80% marketable heads, had a damage rating of 1.3 and a mean of 0.33 DBM larvae and pupae per plant.

Table 1. Head Cabbage Variety Trial. Feeding preference of diamondback moth among 39 varieties of head cabbage. Percent marketable heads of each variety. Kula, Maui, Hawaii. March 29, 1995

Variety	Relative maturity[1]	% Marketable[2]
Heads Up	Early	100 a
Golden Cross	Early	100 a
Pacifica	Early	96.7 ab
Tropical Delight	Late	93.3 ab
Eastern Riches	Late	90.0 abc
Good Seasons	Early	90.0 abc
Fast Vantage	Early	90.0 abc
Stonehead	Early	83.3 bcd
Genuine	Late	80.0 bcde
Blue Thunder	Late	80.0 bcde
Scorpio	Early	80.0 cdef
Gourmet Y.R. 11646S	Late	80.0 cdefg
Charmant	Early	76.7 cdefg
Sure Vantage	Late	73.3 cdefg
Summer Summit	Late	73.3 cdefgh
Blue Gem	Late	73.3 cdefghi
Royal Vantage	Late	70.0 defghi
Savoy Ace	Late	70.0 defghi
Coleguard	Late	60.0 defghij
Superette	Late	66.7 defghijk
Blooming Springs	Late	60.0 defghijk
Fortress	Late	63.3 defghijk
Conquest	Late	56.7 efghijkl
Cheers	Late	60.0 efghijkl
Blue Vantage	Late	60.0 fghijkl
Bravo	Late	50.0 fghijklm
Summer Autumn	Late	56.7 ghijklm
Head Start	Early	50.0 hijklmn
Greenboy	Late	50.0 hijklmn
Custodian	Late	46.7 ijklmno
Arena	Late	50.0 jklmnop
Spring Light	Early	40.0 klmnop
Supreme Vantage	Late	40.0 lmnop
Summit	Early	33.3 mnopq
Market Prize	Late	26.7 nop
Applause	Late	26.7 nopq
Tastie	Early	30.0 opq
Cavalier	Late	26.7 pq
Green Cup	Late	16.7 q

[1]Average dates from transplant
Early cultivars = 50–70 days
Late cultivars = 71–90 days

[2]Means followed by a different letter are significantly different at P≤ 0.0001

Conclusion

The top 12 cultivars that produced a 80% or more marketable heads were: 'Heads up', 'Golden Cross', 'Pacifica', 'Tropical Delight', 'Eastern Riches', 'Good Seasons', 'Fast Vantage', 'Stonehead', 'Genuine', 'Blue Thunder', 'Scorpio' and 'Gourmet Y.R. 11646S. Plant growth factors appear to have no influence on the feeding preference of the DBM. For example, 'Scorpio' is considered a tough, dry cabbage, but it produced a significantly lower percentage of marketable heads than 'Pacifica', a soft, succulent cabbage. Also, the date of maturity appears to have little influence on the percent of marketable heads, since five of the top 12 varieties were late maturing types. In Hawaii, USA, growers prefer to use early varieties over late maturing types due to the additional inputs needed to grow and protect the crop against cabbage pests, as well as tying up the land for an additional 2 to 3 weeks. The results of this study on the feeding preference of the DBM coincide with observations made in commercial fields where different varieties were grown under similar conditions with similar pest control strategies. As the DBM epidemic increased over the past several years, more growers have changed from 'Tastie', a soft, succulent

Table 2. Head Cabbage Variety Trial. Mean number of diamondback moth larvae and pupae per plant at harvest. Kula, Maui, Hawaii. March 29, 1995

Variety	Relative maturity	Mean number of DBM larvae & pupae/plant
Green Cup	Late	5.63 a
Cavalier	Late	2.53 b
Applause	Late	2.40 bc
Supreme Vantage	Late	2.13 bc
Greenboy	Late	2.17 bc
Cheers	Late	1.83 bcd
Superette	Late	2.07 bcde
Tastie	Early	1.67 bcdef
Bravo	Late	1.70 bcdefg
Blue Gem	Late	1.63 bcdefg
Coleguard	Late	1.57 bcdefg
Fortress	Late	1.37 bcdefg
Summer Autumn	Late	1.37 bcdefg
Head Start	Early	1.33 bcdefg
Summit	Early	1.40 bcdefg
Custodian	Late	1.33 bcdefg
Spring Light	Early	1.30 bcdefg
Blue Vantage	Late	1.27 bcdefg
Royal Vantage	Late	1.30 bcdefg
Savoy Ace	Late	1.07 bcdefg
Market Prize	Late	1.10 bcdefg
Arena	Late	1.07 bcdefg
Good Seasons	Early	1.13 bcdefg
Gourmet Y.R. 11646S	Late	1.03 bcdefg
Summer Summit	Late	0.97 bcdefg
Conquest	Late	1.30 bcdefg
Blue Thunder	Late	0.90 bcdefg
Tropical Delight	Late	0.77 bcdefg
Blooming Springs	Late	0.67 cdefg
Stonehead	Early	0.70 cdef
Eastern Riches	Late	0.60 cdef
Fast Vantage	Early	0.57 defg
Pacifica	Early	0.52 defg
Sure Vantage	Late	0.43 defg
Charmant	Early	0.33 efg
Scorpio	Early	0.33 efg
Golden Cross	Early	0.27 fg
Genuine	Late	0.23 fg
Heads Up	Early	0.20 g

Means followed by a different letter are significantly different (Tukey's studentized range test P<0.0001 SAS Institute, version 6.08). Data was transformed by square root (X+0.5) prior to analysis.

cabbage, to 'Scorpio', a tough, dry cabbage. These data suggest that planting varieties that are not preferred by the DBM is an important component in an integrated pest management program for head cabbage.

Table 3. Head Cabbage Variety Trial. Extent of feeding injury to heads by diamondback moth at harvest. Kula, Maui, Hawaii. March 29, 1995

Variety	Relative maturity	Damage rating[1]
Green Cup	Late	2.63 a
Cavalier	Late	2.63 a
Supreme Vantage	Late	2.57 a
Arena	Late	2.53 ab
Tastie	Early	2.37 abc
Coleguard	Late	2.37 abc
Custodian	Late	2.33 abcd
Applause	Late	2.27 abcde
Cheers	Late	2.17 abcdef
Spring Light	Early	2.17 abcdef
Head Start	Early	2.17 abcdef
Stonehead	Early	2.07 abcdefg
Blue Vantage	Late	2.03 abcdefgh
Market Prize	Late	2.00 abcdefgh
Summit	Early	1.87 abcdefghi
Blue Gem	Late	1.83 abcdefghij
Superette	Late	1.80 abcdefghijk
Greenboy	Late	1.80 abcdefghijk
Conquest	Late	1.76 abcdefghijkl
Bravo	Late	1.73 abcdefghijklm
Summer Autumn	Late	1.73 abcdefghijklm
Fortress	Late	1.63 bcdefghijklm
Blue Thunder	Late	1.47 cdefghijklmn
Sure Vantage	Late	1.40 defghijklmn
Savoy Ace	Late	1.37 efghijklmn
Royal Vantage	Late	1.37 efghijklmn
Gourmet Y.R. 11646S	Late	1.33 fghijklmn
Scorpio	Early	1.30 fghijklmn
Summer Summit	Late	1.27 fghijklmn
Charmant	Early	1.23 ghijklmn
Fast Vantage	Early	1.17 ghijklmn
Good Seasons	Early	1.13 hijklmn
Tropical Delight	Late	1.07 ijklmn
Golden Cross	Early	0.93 jklmn
Heads Up	Early	0.90 klmn
Pacifica	Early	0.87 lmn
Genuine	Late	0.83 nm
Eastern Riches	Late	0.70 n

[1]Ranking scale of 0 to 5 (no damage to extensive damage). Means followed by a different letter are significantly different at $P \leq 0.0001$

The effect of stress on the susceptibility of *Plutella xylostella* to *Bacillus thuringiensis*

T. H. Schuler & H. F. van Emden

School of Plant Sciences, University of Reading, Reading RG6 2AS, United Kingdom

Abstract

The following stress factors were tested for their effect on the susceptibility of *Plutella xylostella* (L.) to *Bacillus thuringiensis* Berliner: (a) exposure to low doses of the synthetic pyrethroid cypermethrin, (b) treatment with low doses of the secondary plant compound, azadirachtin and (c) development on partially resistant cabbage cultivars. *B. thuringiensis*-related mortality of cypermethrin- and azadirachtin-stressed larvae did not differ significantly from that of non-stressed larvae. However, larvae reared on different cabbage cultivars differed in their response to *B. thuringiensis*. LC_{50} values of larvae reared on some resistant cultivars were significantly lower than LC_{50} values of larvae reared on susceptible cultivars. However, not all resistant cultivars caused this effect. Although stress treatment with resistant cultivars affected consumption of *B. thuringiensis*-treated leaf discs, these differences in feeding rate did not explain the observed differences in mortality.

Key words: *Bacillus thuringiensis*, stress, azadirachtin, cypermethrin, plant resistance

Introduction

Microbial insecticides based on *Bacillus thuringiensis* Berliner play an increasing role in the control of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), especially as an essential part of integrated pest management (IPM) programmes (Talekar, 1992). The present study investigated the influence of three IPM-related stress factors with different modes of action on the susceptibility to *B. thuringiensis* of this important pest. Two stress factors were insecticides with very different modes of action: the pyrethroid cypermethrin and azadirachtin, a secondary compound of the neem tree (*Azadirachta indica* A. Juss). The third stress factor consisted of cabbage cultivars with partial plant resistance to *P. xylostella*.

Materials and Methods

Stress factors

Aqueous dilutions of Ambush C (10% a.i. cypermethrin, Zeneca Agrochemicals, Fernhurst) were applied to larvae in a Potter tower at a rate of 2 ml per replicate. AZA Powder (10% a.i. azadirachtin, Cardiff Chemicals Ltd., Cardiff) diluted in distilled water containing 0.05% Triton-X-100 was applied as a leaf-dip (Verkerk and Wright, 1993). The insects were treated with *B. thuringiensis* 24 h after those stress factors to minimize the effect of the stress treatments on Dipel consumption and to avoid problems with possible incompatibility. Larval weight and pupation were recorded as indicators for the effect of the stress treatment on larval development *(Table 1)*.

The following field grown cabbage cultivars with varying degrees of resistance to *P. xylostella* (Abro and Wright, 1989) were used to rear larvae: *Brassica oleracea* var. *sabauda* L. Aquarius F1, *B. oleracea* var. *capitata* L. Offenham 2 - Flower of Spring (Offenham) and Minicole F1. Two additional cabbage cultivars were grown in the glasshouse: *B. chinensis* var. *pekinensis* (Rubr.) Sun. Wong Bok and *B. oleracea* var. *capitata* Red Drumhead.

Insects and bioassays

P. xylostella (Rothamsted strain) larvae were reared on Chinese cabbage Wong Bok for tests with cypermethrin and azadirachtin. For bioassays with resistant host plants, larvae were reared from the first instar on the different cultivars. All experiments were carried out at 20˚C. Aqueous serial dilutions of Dipel WP (Abbott Laboratories, Chicago; 16 000 IUP/mg; *B. thuringiensis* subspecies *kurstaki* HD-1), containing 0.05% Triton-X-100, were applied to both sides of Chinese cabbage leaf discs (2.5 cm diam., 2 µl/cm^2). Five to seven concentrations were used in each LC_{50} study with 50 larvae per concentration. Batches of ten third instar larvae received Dipel-treated leaf discs which were left with the larvae for 24 h. After Dipel-administration, the treated leaf discs were exchanged for untreated Chinese cabbage leaf discs with the exception of the second and the third experiment with resistant cultivars *(Table 2)*. In those experiments larvae were provided with untreated leaf discs of the original cultivar for two and four days, respectively. Mortality was assessed after five days.

The small size of the larvae and the strong antifeedant effect of Dipel made it impossible to standardise the amount of Dipel consumed by larvae across treatments. The effect of stress treatments on actual Dipel dose consumed was therefore assessed by measuring the leaf area of Dipel-treated leaf discs remaining at the end of the Dipel treatment.

Table 1. The effect of stress caused by low doses of cypermethrin and azadirachtin on the susceptibility of *P. xylostella* to *B. thuringiensis*

Stress treatment (ppm a.i.)	LC_{50} mg/l (95% confidence limits)	χ^2 for position (df)	Mortality due to stress treatment alone (%)	Larval weight at start of Dipel treatment (mg)[a]	Pupation in controls 10 days after stress treatment (%)[a]
Cypermethrin					
0	9.03 (7.07-11.55)	4.12 (2)	0	1.728b	–
0.1	10.40 (8.14-13.32)		0	1.608b	–
1.0	6.76 (4.86-9.42)		12	0.998a	–
Azadirachtin					
0	9.28 (7.02-12.04)	4.42 (2)	6	1.208a	64.2b
0.2	8.89 (6.42-11.99)		18	1.216a	34.8a
2.0	6.29 (4.57-8.41)		14	1.143a	10.9a

[a]Means followed by a common letter did not differ significantly.

Table 2. The effect of stress caused by resistant cabbage cultivars on the susceptibility of *P. xylostella* to *B. thuringiensis*

Experiment	Cabbage cultivar	Relative degree of plant resistance[a]	LC_{50} mg/l (95% confidence limits)	χ^2 for position (df)
1	Wong Bok	highly susceptible	5.55 (4.48–6.87)	7.20 (2)
	Offenham	susceptible	4.52 (3.61–5.63)	
	Aquarius F_1	intermediate	7.05 (5.51–8.97)	
2	Offenham	susceptible	5.29 (3.46–7.21)	7.93 (1)
	Minicole F_1	highly resistant	2.08 (0.94–3.79)	
3	Wong Bok	highly susceptible	22.05 (18.21–26.84)	24.16 (1)
	Red Drumhead	resistant	11.00 (8.94–13.44)	

[a]Ranking is based on Abro and Wright (1989) and Schuler (1995) for field and glasshouse grown cultivars, respectively.

Data Analysis

Mortality data were analyzed using a logistic model in the statistics package MLP, testing for differences between LC_{50} values and regression slopes with x^2 tests for parallelism and position. LC_{50} values are based on parallel regression since there was no significant difference between slopes. Correction for control mortality followed the procedure described in Finney (1971) which maximises the likelihood function directly and is independent of the level of control mortality. Arcsin transformed (arcsin $\sqrt{(x/100)}$) percentage consumption and pupation data as well as untransformed larval weight data were subjected to analysis of variance carried out by the GLM procedure in SAS.

Results

Mortality data

There was no significant difference between LC_{50} values of cypermethrin-stressed and unstressed larvae, despite a significant decrease in larval weight *(Table 1)*. Low doses of the pyrethroid cypermethrin did, therefore, not have a synergistic effect on *B. thuringiensis*. Azadirachtin-stressed larvae and non-stressed larvae also did not differ significantly in their response to the bacterium *(Table 1)*.

In contrast, partially resistant host plants were found to have a significant effect on the susceptibility of *P. xylostella* to *B. thuringiensis*. x^2 values for position indicated a significant difference between LC_{50} values for all three experiments *(Table 2)*. The first experiment demonstrated an antagonistic effect of the Savoy cabbage Aquarius F_1 on the Dipel-related mortality of *P. xylostella* when compared to the mortality among larvae reared on the susceptible cultivars Wong Bok and Offenham. The following two experiments however, showed that resistant cultivars can increase the susceptibility of *P. xylostella* to *B. thuringiensis.* Larvae reared on the white cabbage Minicole F_1, the most resistant cultivar, were more than twice as susceptible to *B. thuringiensis* than larvae reared on the susceptible white cabbage Offenham. The red cabbage Red Drumhead had the same effect as Minicole F_1 with a significantly lower LC_{50} value than the susceptible Chinese cabbage Wong Bok.

Dipel consumption

The lack of synergism by azadirachtin cannot be attributed to a reduced Dipel intake by stressed larvae since only minor differences in feeding rate of Dipel-treated leaf discs were observed *(Figure 1a)*. Resistant host plants, however, had a stronger effect on the feeding rate, as shown here for Red Drumhead in the last experiment *(Figure 1b)*. Larvae reared on Red Drumhead actually consumed less Dipel-treated leaf than those reared on the susceptible Wong Bok. Differences in Dipel intake do, therefore, not explain the increase in mortality but rather emphasise it.

Discussion

The susceptibility of *P. xylostella* to *B. thuringiensis* was unaffected by any cypermethrin-related

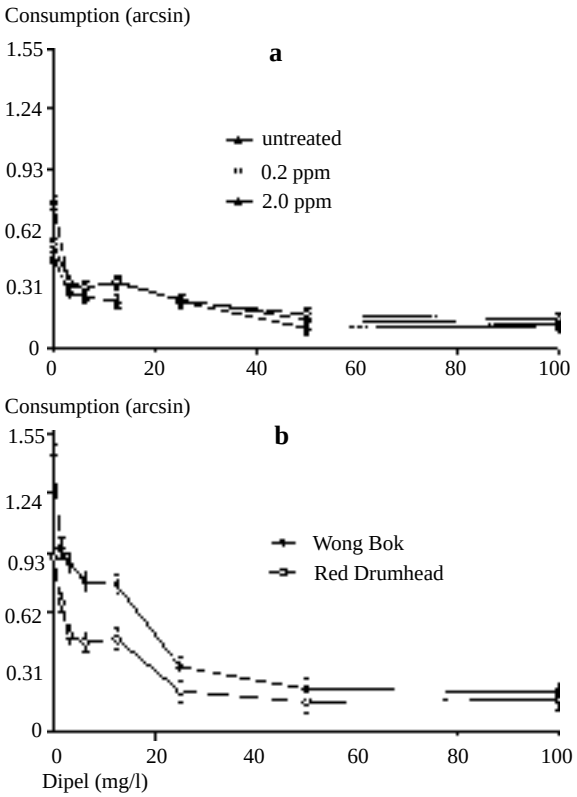


Figure 1. Effect of stress treatment on consumption of Dipel-treated leaf discs of (a) larvae treated with low doses of azadirachtin and (b) larvae reared on susceptible (Wong Bok) and resistant (Red Drumhead) host plants

physiological changes in the insect. Besides their primary action on insect nerves, sublethal doses of pyrethroids have been reported to also cause changes in amine levels, especially octopamine (Hirashima and Eto, 1993), in water excretion (Holden, 1979) and in levels of various amino acids (Jabbar and Strang, 1985). The combination of these sublethal effects resulted in a reduced weight of highly stressed larvae but did not lead to a significantly higher Dipel-related mortality.

The botanical insecticide azadirachtin demonstrated the same lack of effect as cypermethrin. Several effects of azadirachtin on insects would have seemed likely to interact with *B. thuringiensis*: Azadirachtin is known (a) to inhibit trypsin (Timmins and Reynolds, 1992), (b) to reduce the rate of food passage through the insect gut (Mordue (Luntz) *et al.*, 1985), (c) to negatively affect midgut cells (Nasiruddin and Mordue (Luntz), 1993) and (d) to reduce the immune reactivity of the haemolymph (Azambuja *et al.*, 1991). However, the result confirmed the conclusions from a previous field study where a mixture of Dipel and neem extract did not reduce plant damage by *P. xylostella* any more than the single components (Schmutterer, 1992). This second stress factor thus again provided no indication that general physiological stress affects the susceptibility of *P. xylostella* to *B. thuringiensis*. Previous work with *Spodoptera exigua* (Hübner) showed an antagonistic effect of neem extract on *B. thuringiensis* (Moar and Trumble, 1987). Moar and Trumble (1987) used a neem extract concentration which killed 37% of *S. exigua* larvae and they suggested that the negative effect might have been caused by a reduced intake of *B. thuringiensis* due to antifeedant and growth-regulation effects of azadirachtin. Azadirachtin doses in the present investigation were considerably lower and consumption was virtually unaffected in the various Dipel treatments.

The results presented here demonstrate for the first time that the effect of *B. thuringiensis* on *P. xylostella* can be influenced by the host plant. The mechanisms by which resistant cabbage cultivars enhance *B. thuringiensis*-related mortality are unclear since the resistance factors have not been identified and there are a multitude of possible sites of interaction within the insect. General physiological stress to insects caused by poor host plants has been suggested as one factor involved in enhancing the effect of insect pathogens (Hare and Andreadis, 1983; Richter *et al.*, 1987) but seems unlikely since none of the other stress factors produced a similar effect. Earlier studies have already shown that resistant cabbage cultivars can increase the susceptibility of *P. xylostella* to the insecticides cypermethrin and abamectin (Abro and Wright, 1989) and improve the control by parasitoids (Bogahawatie, 1993) and predators (Eigenbrode *et al.*, 1995).

Individual secondary plant compounds have been demonstrated to affect *B. thuringiensis in vivo*. Some have been implicated in an increase in *B. thuringiensis* efficacy, e.g. L-canavanine in *Manduca sexta* (L.) (Felton and Dahlman, 1984) and tomatine in *Trichoplusia ni* (Hübner) (Reichelderfer, 1991), while others had negative effects, e.g. nicotine in *M. sexta* (Krischik *et al.*, 1988) and tannins in *Heliothis virescens* (Forskal) (Navon *et al.*, 1993). Suggested mechanisms were alteration of gut pH, formation of complexes with ∂-endotoxin, effects on midgut permeability and inhibition of vegetative growth of the bacterium. The latter could be an important effect, since *B. thuringiensis* HD-1 spores increase the efficacy of ∂-endotoxins in *P. xylostella* and viable cells of the bacterium have been found in the haemolymph of larvae as soon as two days after treatment (Miyasono *et al.*, 1994).

Proteases play an important part in the pathology of *B. thuringiensis* and protease inhibitors have been found to synergise *B. thuringiensis* in several lepidopteran species (MacIntosh *et al.*, 1990). Seed extracts of mustard and oilseed rape also significantly enhanced *B. thuringiensis*-related mortality of *H. virescens*, an activity which MacIntosh *et al.* attributed to protease inhibitors in the seeds. Leaves of brassicas do possess tryptic inhibitory activity which varies considerably between species and cultivars (Broadway, 1989). However, protease inhibitors did not affect *B. thuringiensis*-related mortality of *P. xylostella* in a study by Tabashnik *et al.* (1992).

More research is needed to clarify the effect of resistant cabbage cultivars on *B. thuringiensis* but this additional benefit of host plant resistance should be exploited in IPM programmes.

References

Abro, G. H. and Wright, D. J. (1989). Host plant preference and the influence of different cabbage cultivars on the toxicity of abamectin and cypermethrin against *Plutella xylostella* Lepidoptera: Plutellidae. *Annals of Applied Biology* 115 : 481–487.

Azambuja, P., Garcia, E. S., Ratcliffe, N. A. and Warthen, J., Jr. (1991). Immune-depression in *Rhodnius prolixus* induced by the growth inhibitor, azadirachtin. *Journal of Insect Physiology* 37 : 771–777.

Bogahawatie, C. N. L. (1993). Glasshouse and Field Studies of Diamondback Moth *Plutella xylostella* (Lepidoptera: Yponomeutidae) on Host Plant Resistance in Brassicas and Biological Control. PhD Thesis, University of Reading, 213 pp.

Broadway, R. M. (1989). Tryptic inhibitory activity in wild and cultivated crucifers. *Phytochemistry* 28 : 755–758.

Eigenbrode, S. D., Moodie, S. and Castagnola, T. (1995). Predators mediate host plant resistance to a phytophagous pest in cabbage with glossy leaf wax. *Entomologia Experimentalis et Applicata*, 77 : 335–342.

Felton, G. W. and Dahlman, D. L. (1984). Allelochemical induced stress: effects of L-canavanine on the pathogenicity of *Bacillus thuringiensis* in *Manduca sexta*. *Journal of Invertebrate Pathology* 44 : 187–191.

Finney, D. J. (1971). Probit Analysis. 3rd ed., Cambridge University Press, Cambridge, 333 pp.

Hare, J. D. and Andereadis, T. G. (1983). Variation in the susceptibility of *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) when reared on different host plants to the fungal pathogen, *Beauveria bassiana*, in the field and laboratory. *Environmental Entomology* 12 : 1891–1896.

Hirashima, A. and Eto, M. (1993). Chemical stress-induced changes in the biogenic amine levels of *Periplaneta americana* L. *Pesticide Biochemistry and Physiology* 46 : 131–140.

Holden, J. S. (1979). Absorption and metabolism of permethrin and cypermethrin in the cockroach and the cotton-leafworm larvae. *Pesticide Science* 10 : 295–307.

Jabbar, A. and Strang, R. H. C. (1985). The effects of chemical and physical stress on the concentrations of amino compounds in the haemolymph and nervous system of locusts and cockroaches. *Journal of Insect Physiology* 31: 359–370.

Krischik, V. A., Barbosa, P. and Reichelderfer, C. F. (1988). Three trophic level interactions: allelochemicals, *Manduca sexta* (L.), and *Bacillus thuringiensis* var. *kurstaki* Berliner. *Environmental Entomology* 17: 476–482.

MacIntosh, S. C., Kishore, G. M., Perlak, F. J., Marrone, P. G. and Stone, T. B. (1990). Potentiation of *Bacillus thuringiensis* insecticidal activity by serine protease inhibitors. *Journal of Agricultural Food Chemistry* 38: 1145–1152.

Miyasono, M., Inagaki, S., Yamamoto, M., Ishiguro, T., Takeda, R. and Hayashi, Y. (1994). Enhancement of delta-endotoxin activity by toxin-free spore of *Bacillus thuringiensis* against the diamondback moth, *Plutella xylostella*. *Journal of Invertebrate Pathology* 63 : 111–112.

Moar, W. J. and Trumble, J. T. (1987). Toxicity, joint action, and mean time of mortality of Dipel 2X, avermectin B_1, neem, and thuringiensin against beet armyworms (Lepidoptera: Noctuidae). *Journal of Economic Entomology* 80 : 588–592.

Mordue (Luntz), A. J., Cottee, P. K. and Evans, K. A. (1985). Azadirachtin: its effect on gut motility, growth and moulting in *Locusta*. *Physiological Entomology* 10: 431–437.

Nasiruddin, M. and Mordue (Luntz), A. J. (1993). The effect of azadirachtin on the midgut histology of the locusts, *Schistocerca gregaria* and *Locusta migratoria*. *Tissue and Cell* 25: 875–884.

Navon, A., Hare, J. D. and Federici, B. A. (1993). Interactions among *Heliothis virescens* larvae, cotton condensed tannin and the CryIA(c) delta-endotoxin of *Bacillus thuringiensis*. *Journal of Chemical Ecology* 19: 2485–2499.

Reichelderfer, C. F. (1991). Interactions among allelochemicals, some Lepidoptera, and *Bacillus thuringiensis* Berliner. In *Microbial Mediation of Plant-Herbivore Interactions* (ed. P. Barbosa *et al.*) pp. 507–524. John Wiley, Chichester.

Richter, A. R., Fuxa, J. R. and Abdel-fattah, M. (1987). Effect of host plant on the susceptibility of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to a nuclear polyhedrosis virus. *Environmental Entomology* 16: 1004–1006.

Schmutterer, H. (1992). Control of diamondback moth by application of neem extracts. *Proceedings of the 2nd International Workshop on Diamondback Moth and Other Crucifer Pest*s Tainan 1990, pp. 325–332.

Schuler, T. H. (1995). Laboratory Studies on the Effect of 'Stress' on the Susceptibility of the Diamondback Moth (*Plutella xylostella*) and the Rose-Grain Aphid (*Metopolophium dirhodum*) to Insect Pathogens. PhD Thesis, University of Reading, 255 pp.

Tabashnik, B. E., Finson, N. F. and Johnson, M. W. (1992). Two protease inhibitors fail to synergize *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). *Journal of Economic Entomology* 85 : 2082–2087.

Talekar, N. S. (ed.) (1992). Diamondback Moth and Other Crucifer Pests. *Proceedings of the 2nd International Workshop on Diamondback Moth and Other Crucifer Pest*s Tainan 1990, 603 pp.

Timmins, W. A. and Reynolds, S. E. (1992). Azadirachtin inhibits secretion of trypsin in midgut of *Manduca sexta* caterpillars: reduced growth due to impaired protein digestion. *Entomologia Experimentalis et Applicata* 63 : 47–54.

Verkerk, R. and Wright, D. J. (1993). Biological activity of neem seed kernel extracts and synthetic azadirachtin against larvae of *Plutella xylostella*. *Pesticide Science* 37 : 83–91.

Inheritance and stability of resistance to *Bacillus thuringiensis* formulations in field population of diamondback moth, *Plutella xylostella*

Katsuki Imai and Yuhzoh Mori

Pesticide Section, Agricultural R & D Center, ZEN-NOH, 5-5-1 Higashi-Yahata, Hiratsuka, Kanagawa 254, Japan

Abstract

Levels, genetic traits and stability of resistance to *Bacillus thuringiensis* of the Bang Bua Tong (BS) population of the diamondback moth *Plutella xylostella* collected in Thailand were investigated. The BS population exhibited 668 and 66.5-fold resistance to formulations derived from *B. thuringiensis* subsp. *kurstaki* (Toarow CT®) and *B. thuringiensis* subsp. *aizawai* (XenTari®), respectively. In order to determine the genetic traits of resistance to *B. thuringiensis* subsp. *kurstaki*, two reciprocal crosses and backcross were conducted using a Japanese susceptible (S) population, BS population. Analysis of dose-mortality relationship of the S population, BS population, F_1 progenies of the two reciprocal crosses and backcross progenies suggested that resistance to *B. thuringiensis* subsp. *kurstaki* in the moth might be controlled by some incompletely recessive autosomal alleles. Resistance to *B. thuringiensis* subsp. *kurstaki* and *aizawai* in the moth have remained at high levels despite rearing in the absence of *B. thuringiensis* selection.

Key words: *Bacillus thuringiensis*, insecticide resistance, genetics, *Plutella xylostella*.

Introduction

Diamondback moth, *Plutella xylostella*, has become one of the most difficult insect pests to control in the world because of the development of resistance to organophosphorus, carbamates, synthetic pyrethroids insecticides and insect growth regulators (Tabashnik *et al.*, 1987; Shelton and Wyman, 1992; Shelton *et al*., 1993; Furlong and Wright, 1994). Especially in southeast Asian countries, this increased resistance to insecticides causes a major problem (Cheng *et al*., 1992; Syed, 1992, Sinchaisri *et al*., 1995).

Microbial insecticide, *Bacillus thuringiensis* is highly toxic to certain pests, but it has little or no toxicity to most beneficial insects and other nontarget organisms (Flexner *et al*., 1986). Therefore *B. thuringiensis* has become a major component of pest management and is the most widely used bioinsecticide to control and diamondback moth. However, increased use of *B. thuringiensis* intensified selection for resistance in the field. In fact, cases of development of resistance to *B. thuringiensis* in the field populations of diamondback moth were documented (Tabashnik *et al*., 1990; Hama *et al*., 1992; Martinez-Ramirez *et al*., 1995). Resistance to *B. thuringiensis* in diamondback moth is associated with reduced binding of toxins to midgut membranes (Ferre *et al.*, 1991) and processing of *B. thuringiensis* toxins by trypsin or trypsinlike proteases is not an important mechanism of resistance (Tabashnik *et al.*, 1992). Furthermore, resistance to *B. thuringiensis* in diamondback moth is inherited as an autosomal recessive trait which is inherently unstable (Hama *et al.*, 1992; Tabashnik *et al.*, 1992). Furthermore, resistance to *B. thuringiensis* in diamondback moth is inherited as an autosomal recessive trait which is inherently unstable (Hama *et al.*, 1992; Tabashnik *et al.*, 1992). Recent report suggested that at least one genotype conferring resistance to *B. thuringiensis* in diamondback moth existed (Tabashnik *et al.*, 1995). Also, reports on physiological and behavioral responses to *B. thuringiensis* in diamondback moth suggested that resistance had a physiological basis (Schwartz *et al.*, 1991).

Knowledge of the genetic basis of resistance to *B. thuringiensis* is useful for successful management of resistance. Also detection, monitoring and risk assessment of resistance can benefit greatly from information about its mode of inheritance.

We report here results on studies of inheritance and stability of resistance to *B. thuringiensis* formulations in field population of diamondback moth from Thailand which had never been subjected to laboratory selection.

Materials and Methods

Insects Susceptible (S) population and *B. thuringiensis* resistant (BS) population were used in this study. The S population provided by Hokko Chemical Industry Co., Ltd. in 1980, had been maintained in the laboratory without exposure to insecticides for >200 genetations. The resistant BS population were collected in 1994 from a radish farm at Bang Bua Thong in Thailand. This farm had been treated frequently with commercial formulations of *B.*

thuringiensis subsp. *kurstaki* and *aizawai*. The BS population had been maintained in the laboratory in the absence of *B. thuringiensis* selection. Larvae were reared on germinating rape seeds in the laboratory at 25±2 °C with a photoperiod of 16L:8D (Koshihara and Yamada, 1976). Resistance to *B. thuringiensis* in BS population remained at high levels within 17 generations in the absence of selection. Insects used in the genetics experiments were obtained from the generation after the field.

To obtain F_1 hybrid offspring and backcross offspring, pupae were individually isolated in a glass tube (0.8 cm diameter x 5 cm height). After adult eclosion, female or male adults were distinguished, then males and females from the respective population were mass-crossed in a screened cage (35 x 35 x 30 cm). In first experiment with F_1 hybrids, the offsprings from each reciprocal cross were tested (BS♀ x S♂ and S♀ x BS♂). No difference was observed between the two reciprocal crosses, thus, backcross offsprings were obtained from F_1 (S♀ x BS♂) ♀ x BS♂. Third-instar were used for toxicity assays.

Insecticides The following *B. thuringiensis* formulations were used for the toxicity assays: ToarowCT® (7.0% *B. thuringiensis* subsp. *kurstaki* crystal toxin WP) provided by Toagosei Chemical Ind., Ltd., Thuricide® (10.0% *B. thuringiensis* subsp. *kurstaki* crystal toxin with spores WP) provided by SDS Biotech K.K., Dipel® (10.0% *B. thuringiensis* subsp. *kurstaki* crystal toxin with spores WP) provided by Hokko Chemical Industry Co., Ltd., Bacilex® (10.0% *B. thuringiensis* subsp. *kurstaki* and *aizawai* crystal toxin with spores WP) provided by Shionogi & Co., Ltd., XenTari® (10.3% *B. thuringiensis* subsp. *aizawai* crystal toxin with spores WG) provided by Tomen Corporation. Concentration of these *B. thurigiensis* formulations are expressed in the weight (mg) of formulations per volume (l) of solution.

Toxicity assays The leaf dip method was used for toxicity assays. A piece of cabbage leaf (approx. $35cm^2$) cut from fully expanded leaves grown in the greenhouse was dipped for 1 min. in insecticide solutions which were prepared by diluting *B. thuringiensis* formulations with 0.02% of a spreader, Neo-Esterin® (Kumiai Chemical Industry Co., Ltd.). After being air-dried, leaves were put in a plastic vessel (9 cm diameter x 5 cm height). Then, third-instar larvae were added to the vessel and kept at 25±2 °C with a photoperiod of 16L:8D for 72h before they were checked for mortality. Five or more concentrations of insecticide plus a distilled water control were evaluated using 10 larvae per concentration in each test. Each test was replicated 3 times. Results were analyzed by probit analysis (Bliss, 1935) to obtain LC_{50} and LC_{95} values.

Results

Susceptibility of B. thuringiensis

Susceptibilities of S population and BS population to five *B. thuringiensis* formulations is shown in *Table 1*. The LC_{50} values of the resistant BS population to ToarowCT®, Thuricide®, Dipel®, Bacilex® and XenTari" were 688, 731, 161, 45.0, 66.5 times greater than the LC_{50} values of the susceptible S population, respectively. Resistance levels to ToarowCT®, Thuricide® and Dipel® were high, but moderate, to Bacilex® and XenTari®. These results suggest that BS population exhibit a high level of resistance to *B. thuringiensis* subsp. *kurstaki* but a moderate level to subsp. *aizawai* and their combinations.

Stability of resistance to B. thuringiensis

BS population was reared for 17 generations without exposure to any *B. thuringiensis* in the laboratory. Resistance of BS population to five *B. thuringiensis* formulations fluctuated among the generations, but remained high for 17 generations, indicating that BS population was homogeneous for *B. thuringiensis* resistance *(Fig. 1)*.

Genetic traits of resistance to B. thuringiensis

The LC_{50}s of the reciprocal F_1 crosses between the susceptible and resistant populations did not differ significantly *(Table 2, Fig. 2)*. Thus, maternal effects on resistance were not evident. The similarity between the LC_{50}s of reciprocal F_1 crosses indicates that the genetic basis of resistance to *B. thuringiensis* (ToarowCT®) was autosomal rather than sex-linked. Therefore, the dosage-mortality curve of F_1 calculated from the pooled mortality is presented in *Table 2*. According to the method of Stone (1968), the degrees of dominance in *B. thuringiensis* resistance were calculated to be D= -0.14 at LC_{50}, 0.007 at LC_{95}, suggesting that *B. thuringiensis* resistance in BS population is incompletely recessive.

If *B. thuringiensis* resistance in the BS population is controlled by a single genetic factor on an autosome, then S population is SS, BS population is RR, F_1 progeny is RS and backcross of F_1 x BS population produces progeny that are 50% RR and 50% RS. To confirm whether *B. thuringiensis* resistance in BS population is monogenic, expected mortality calculated from mortality of F_1 and BS population compared with observed mortality of backcross progeny. Expected mortality was calculated as follows: expected percentage F_1 at X=0.5 x (% mortality of F_1 at X + % mortality of BS population at X). Expected mortality from a monogenic model of resistance did not correspond closely to observed backcross mortality *(Table 3)*. Significant deviation was not observed between mortality predicted from monogenic model and observed mortality at 40, 20, 10, 5, 2.5 mg formulation/l ($P>0.05$), however, at 320, 160, 80 mg formulation/l significant deviation was observed ($P<0.01$). Observed mortality of backcross progeny in high concentrations (320, 160, 80 mg formulation/l) were significantly higher than expected mortality. This

Table 1. Susceptibilities of *P. xylostella* to *B. thuringiensis* formulations

Insecticide	Population[a]	LC_{50}, mg formulation/l (95% CL)	Slope	LC_{95}	RR[b]
Toarow CT®	S	0.920 (0.120–7.04)	4.24	2.24	
	BS	615 (354–1070)	1.94	4 331	668
Thuricide®	S	2.76 (1.63–4.68)	2.29	14.4	
	BS	2 018 (1063–3829)	2.27	1 0671	731
Dipel®	S	4.41 (1.35–14.4)	1.07	152	
	BS	708 (374–1342)	1.70	7 262	161
Bacilex®	S	4.60 (2.50–8.40)	1.84	36.1	
	BS	207 (86.7–497)	2.98	739	45.0
XenTari®	S	2.64 (1.30–5.37)	1.67	25.4	
	BS	173 (76.0–391)	1.96	1 187	66.5

[a]BS population were tested on generation 3 after collection
[b]LC_{50} of BS population/LC_{50} of S population

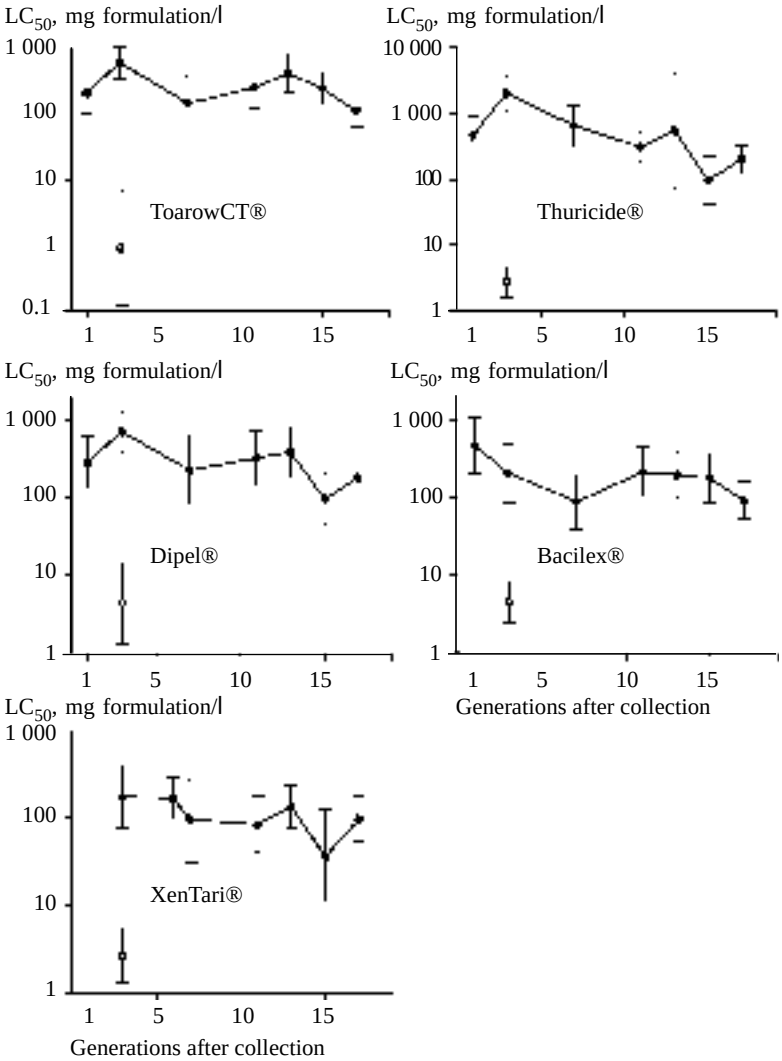


Figure 1. Stability of resistance to five B. thuringiensis formulations in BS population, which was not selected with B. thuringiensis after collection. Vertical bars indicate 95% confidence limit

Table 2. Toxicity of Toarow CT^R to F_1 and backcross larvae of *P. xylostella*

Population[a]	LC_{50}, mg formulation/l (95% CL)	Slope	LC_{95}	RR[b]
Parent				
S	0.402 (0.022–0.730)	2.96	1.45	
BS	256 (146–446)	1.65	2 543	637
F1				
S♀ x BS♂	6.03 (4.04–9.01)	1.50	76.0	15.0
BS♀ x S♂	7.02 (4.75–10.9)	1.52	86.4	17.5
Pooled	6.17 (4.64–8.19)	1.64	62.4	15.3
Backcross				
(S♀ x BS♂)♀ x BS♂	24.3 (15.8–37.4)	2.33	123.7	60.4

[a]Insect used in this genetics experiment were obtain from generations 15 BS population

[b]LC_{50} of BS population/LC_{50} of S population

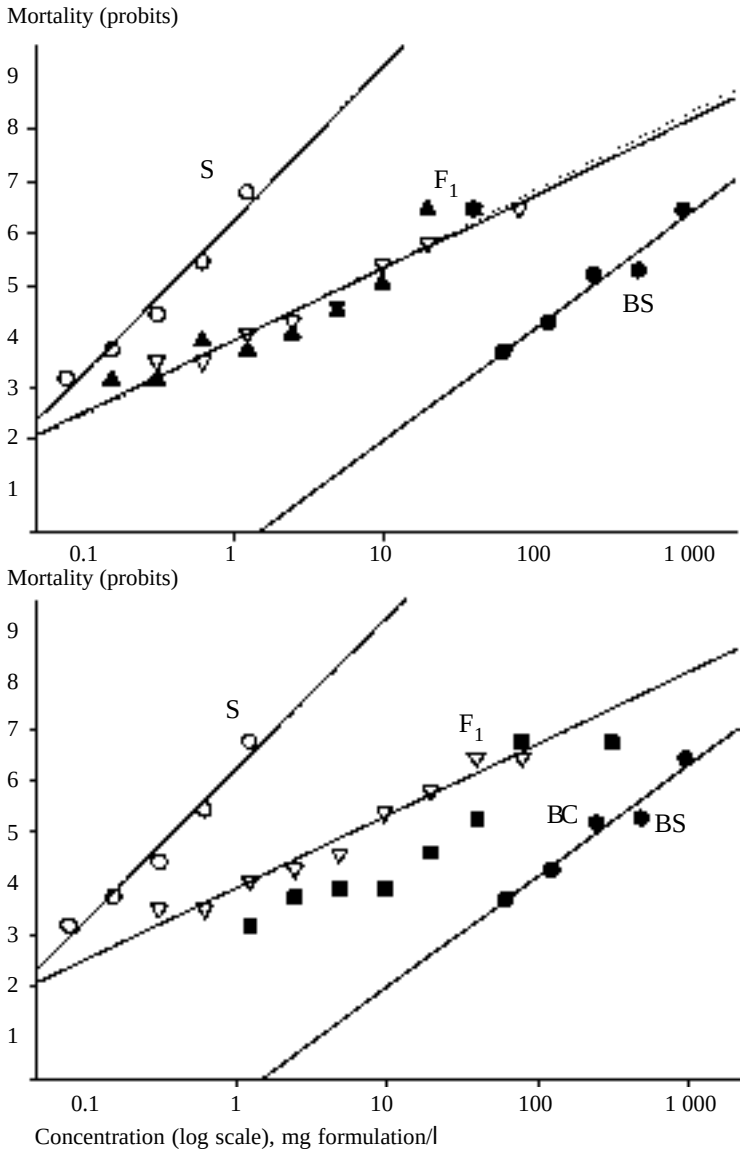


Figure 2. Responses of susceptible (S), resistant (BS), susceptible x resistant (F_1) and F_1 x resistant (BC) larvae of P. xylostella to ToarowCT®. ○ : Susceptible population, ● : BS population, ∇ : F_1; S♀ x BS♂, ▲ : F_1; BS♀ x S♂, ■: BC; F_1 (S♀ x BS♂)♀x BS♂

Table 3. Deviation between observed and expected mortality for a monogenic model

Concn, mg formulation/l	Observed		Expected		χ^2	P
	Dead	Alive	Dead	Alive		
320	1	29	8	52	4.5	<0.01
160	0	30	19	41	9.1	<0.01
80	1	29	26	34	14	<0.01
40	11	19	29	31	1.5	>0.05
20	18	12	35	25	0.05	>0.05
10	24	6	40	20	2.3	>0.05
5	24	6	49	11	0.08	>0.05
2.5	25	5	52	8	0.37	>0.05

[a]Expected % mortality at concentration X = 0.5 x (% mortality of F_1 at X + % mortality of BS population at X)

result suggests that *B. thuringiensis* resistance in BS population may be controlled by some loci.

Discussion

In field populations of diamondback moth, several cases of resistance to *B. thuringiensis* were reported (Tabashnik *et al.*, 1990; Hama *et al.*, 1992; Martinez-Ramirez *et al.*, 1995). Hama *et al.* (1992) documented that the population colected from watercress greenhouse in Japan, where *B. thuringiensis* subsp. *kurstaki* (ToarowCT®) had been applied a total of 40 or 50 times over 3–4 years, exhibited extremely high level of resistance to the formulation derived from *B. thuringiensis* subsp. kurstaki, however low levels of resistance to *B. thuringiensis* subsp. *kurstaki* and *aizawai* combinations (Bacilex®). In Indianmeal moth, *Plodia interpunctella*, resistance to *B. thuringiensis* was specific towards unique constituents of HD-1 type spore-crystal complex rather than general towards all *B. thuringiensis* crystal types (McGaughey and Johnson, 1987). This suggest that cross-resistance to *B. thuringiensis* may be restricted to subspecies of *B. thuringiensis* or crystal toxin. In contrast, the field population of diamondback moth (BS population) collected in 1994 from a radish farm at Bang Bua Thong in Thailand, where commercial formulations of *B. thuringiensis* subsp. *kurstaki* and *aizawai* had been applied frequently, exhibited high level resistance to *B. thuringiensis* subsp. *kurstaki* and *aizawai*. This indicates that diamondback moth has developed resistance to *B. thuringiensis* subsp. *kurstaki* and *aizawai* when both *B. thuringiensis* formulations are used repeatedly in the field.

High levels of resistance to *B. thuringiensis* in BS population have remained within 17 generations in the absence of *B. thuringiensis* selection. The recovery of susceptibility in a resistant population is presumably attributed to the case where the existing frequency of susceptible gene goes up in the population. It is a result of inferior intrinsic rate of natural increase and inferior fitness of resistant population. Furthermore, it largely depends on whether the population is homogeneous or heterogeneous to resistance. However, we have not examined intrinsic rate of natural increase and fitness value of BS population in this experiment.

Analysis of dosage-mortality relationships of two reciprocal crosses offsprings derived from the cross of Japanese S population and BS population has suggested that resistance of BS population to *B. thuringiensis* was incompletely recessive and autosomally inherited. This result coincided with results of Hama *et al.* (1992) and Tabashnik *et al.* (1992), and suggested that *B. thuringiensis* resistance is controlled by an incompletely recessive, autosomal single allele. However, dosage-mortality relationships of backcross offsprings have suggested that resistance of BS population to *B. thuringiensis* might be controlled by some loci. The *B. thuringiensis* resistance population examined by Hama *et al.* (1992) exhibited extremely high level of resistance to the formulation derived from *B. thuringiensis* subsp. *kurstaki*, with, however, low levels of resistance to *B. thuringiensis* subsp. *kurstaki* and *aizawai* combinations. In contrast, BS population exhibited high level resistance to *B. thuringiensis* subsp. *kurstaki* and *aizawai*. Recent report suggested that at least one genotype conferring resistance to *B. thuringiensis* in diamondback moth which was not inherently unstable existed (Tabashnik *et al.*, 1995). In other test, Sim and Stone (1991) reported that laboratory-selected resistance to tobacco budworm, *Helicoverpa virescens*, to 130-kDa delta-endotoxin of *B. thuringiensis* was autosomally inherited, incompletely dominant, and controlled by several loci. In Indianmeal moth, *P. interpunctella*, it is known that resistance was stable when selection was discontinued and inherited as a recessive trait (McGaughey, 1985). In our genetics experiments, we assumed that BS population was possibly homogeneous to resistance because it remained at high levels in the absence of *B. thuringiensis* selection. However, BS population was a field population which was not selected with any *B. thuringiensis* after collection, so it might be heterogeneous to resistance. It is necessary to use population selected by *B. thuringiensis* in the laboratory which is possibly homogeneous to resistance.

BS population exhibited resistance to not only *B. thuringiensis* subsp. *kurstaki* but also *aizawai*. It is necessary to examine genetic traits of resistance to *Bacillus thuringiensis* subsp. *aizawai* in BS population.

Acknowledgement

We gratefully thank Professor T. Miyata of Nagoya University for useful suggestions to our works. Thanks are also extended to related chemical companies for providing insecticide formulations.

References

Bliss, C. I. (1935). The calculation of dosage-mortality curve. *Ann. Appl. Biol.* ***22:*** 134–167

Cheng, E. Y., C. H. Kao and C. S. Chiu (1992). Resistance, cross-resistance and chemical control of diamondback moth in Taiwan: Recent developments. *In:* Diamondback Moth and Other Crucifer Pests (N.S. Talekar, eds.). AVRDC, Shanhua, Taiwan, pp. 465–475

Ferre, J., M. D. Real, J. Van Rie, S. Jansens and M. Peferoen (1991). Resistance to the *Bacillus thuringiensis* bioinsecticide in a field population of *Plutella xylostella* is due to a change in a midgut membrane receptor. *Proc. Natl. Acad. Sci. U.S.A.* ***88:*** 5119–5123

Flexner, J. L., B. Lighthart and B. A. Croft (1986). The effects of microbial pesticides on non-target, beneficial arthropods. *Agric. Ecosyst. Environ.* ***16:*** 203–254

Furlong, M. J. and D. J. Wright (1994). Examination of stability of resistance and cross-resistance patterns to acylurea insect growth regulators in field populations of the diamondback moth, *Plutella xylostella*, from Malaysia. *Pestic. Sci:* ***42:*** 315–326

Hama, H., K. Suzuki and H. Tanaka (1992). Inheritance and stability of resistance to *Bacillus thuringiensis* of the diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Yponomeutidae). *Appl. Entomol. Zool.* ***27:*** 355–362

Koshihara, T. and H. Yamada (1976). A simple mass-rearing technique for the diamondback moth, *Plutella xylostella* (L.), on germinating rape seeds. *Jpn. J. Appl. Entomol. Zool.* ***20:*** 110–114 (in Japanese with English summary)

Martinez-Ramirez, A. C., B. Escriche, M. D. Real, F. J. Silva and J. Ferre (1995). Inheritance of resistance to *Bacillus thuringiensis* toxin in a field population of diamondback moth *(Plutella xylostella). Pestic. Sci.* ***43:*** 115–120

McGaughey, W. H. (1985). Insect resistance to the biological insecticide *Bacillus thuringiensis. Science* ***229:*** 193–195

McGaughey, W. H. and D. E. Johnson (1987). Toxicity of different serotypes and toxins of *Bacillus thuringiensis* to resistant and susceptible Indianmeal moths (Lepidoptera: Pyralidae). *J. Econ. Entomol.* ***80:*** 1122–1226

Schwartz, J. M., B. E. Tabashnik and M. W. Johnson (1991). Behavioral and physiological responses of susceptible and resistant diamondback moth larvae to *Bacillus thuringiensis. Entomol. exp. appl.* ***61:*** 179–187.

Shelton, A. M. and J. A. Wyman (1992). Insecticide resistance of diamondback moth in North America. In Diamondback Moth and Other Crucifer Pests (N.S. Talekar, eds.). AVRDC, Shanhua, Taiwan, pp. 447–454

Shelton, A. M., J. A. Wyman, N. L. Cushing, K. Appelbeck, T. J. Dennehy, S. E. R. Mahr and S. D. Eigenbrode (1993). Insecticide resistance of diamondback moth (Lepidoptera: Plutellidae) in North America. *J. Econ. Entomol.* ***86:*** 11–19

Sim, S. T. and T. B. Stone (1991). Genetic Basis of tobacco budworm resistance to an engineered *Pseudomonas fluorescens* expressing the δ-endotoxin of *Bacillus thuringiensis* kurstaki. *J. Invertebr. Pathol.* ***57:*** 206–210

Sinchaisri, N., T. Miyata, M. Kuwahara, W. Pushtapakorcha, P. Keinmeesuke, T. Saito and A. Vattanatangum (1995). Bioassay of candidate insecticides and monitoring of insecticide of resistance of field collected diamondback moth in 1987–1994. *In: Management of Brown Planthopper and Resistance of Diamondback Moth* (T. Saito, T. Miyata, N. Sinchaisri, eds.). Nagoya University Cooperation Ptress, Chikusa, Nagoya, pp. 56–69

Stone, B. F. (1968). A formula for determining degree of dominance in cases of monofactorial inheritance of resistance to chemicals. *Bull. Wld. Hlth. Org.* ***38:*** 325–326

Syed, A. R. (1992). Insecticide resistance in diamondback moth in Malaysia. In Diamondback Moth and Other Crucifer Pests (N.S. Talekar, eds.). AVRDC, Shanhua, Taiwan, pp. 419–426

Tabashnik, B. E., N. L. Cushing and M. W. Johnson (1987). Diamondback moth (Lepidoptera: Plutellidae) resistance to insecticides in Hawaii: Intra-island variation and cross-resistance. *J. Econ. Entomol.* ***80:*** 1091–1099

Tabashnik, B. E., N. L. Cushing, N. Finson and M. W. Johnson (1990). Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). *J. Econ. Entomol.* ***83:*** 1671–1676

Tabashnik, B.E ., J. M. Schwartz, N. Finson and M. W. Johnson (1992). Inheritance of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). *J. Econ. Entomol.* ***85:*** 1046–1055

Tabashnik, B. E., N. Finson, M. W. Johnson and D. G. Heckel (1995). Prolonged selection affects stability fo resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). *J. Econ. Entomol.* ***8:*** 219–224

Control of cabbage Lepidoptera by naturally occurring arthropod predators

M. A. Schmaedick[1], A. M. Shelton[1], and M. P. Hoffmann[2]

[1]*Department of Entomology, New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456, U.S.A.*

[2]*Department of Entomology, Cornell University, Ithaca, NY 14853, U.S.A.*

Abstract

In recent years, pesticide resistance in some lepidopteran species, along with a general desire to reduce dependence on chemical pesticides, have led to increased interest in evaluating and enhancing the effects of naturally occurring biological control agents on the crucifer Lepidoptera. Due to the relative ease of documenting the impact of parasitoids on pest populations, however, virtually all the research on natural enemies have focused on parasitoids, while the role of predators has remained largely unexplored. Our work evaluating the effects of predatory arthropods on *Pieris rapae* in cabbage shows that generalist predators are responsible for much of the mortality of this pest in our area. We describe predator exclusion experiments and laboratory predation assays that demonstrate the important role of these natural enemies in reducing populations of *P. rapae*. As generalist feeders, these predators undoubtedly affect populations of other crucifer Lepidoptera as well and have the potential to survive in the crop at low pest densities by feeding on alternative prey. These attributes, along with their propensity to destroy the early stages of pests, make these predators potential key players in efforts to increase reliance on non chemical methods for managing crucifer Lepidoptera.

Key words: *Pieris rapae*, predation, cabbage

Introduction

Occurrence of insecticide resistance in populations of lepidopteran pests of cabbage, along with a desire to reduce use of broad spectrum insecticides, have led to increased interest in ecologically based management strategies. Improved understanding of the natural mortality factors affecting populations of Lepidoptera in cabbage is critical to development of such strategies. While numerous studies have addressed the role of insect parasitoids in controlling crucifer Lepidoptera, much less is known about the effects of predators (Jones, 1981; Talekar and Shelton, 1993). *Pieris rapae* L.(Lepidoptera: Pieridae) is one of the most important pests of crucifer vegetables (e.g., cabbage, broccoli, cauliflower) in New York State and many other crucifer growing areas worldwide. We describe initial results of our efforts to estimate the overall mortality of *P. rapae* eggs and larvae due to arthropod predation and to determine the predator species causing the mortality. Overall mortality was estimated by comparing *P. rapae* survivorship on plants from which predators were excluded to that on plants to which arthropod predators were allowed access. Species preying on *P. rapae* were determined by use of sticky traps to ascertain predator species that occur on cabbage plants followed by laboratory predation assays to learn which of these species feed on *P. rapae* early stages.

Materials and Methods

Estimation of mortality from arthropod predation

Mortality of *P. rapae* eggs and larvae due to arthropod predators in cabbage fields was estimated by comparing survivorship on protected and exposed plants infested with known numbers of *P. rapae* eggs. The experiment was conducted in two 0.2 ha cabbage fields planted with "Vantage Point" seedlings on 2–5 June 1995. An additional 120 seedlings were planted into 30.5 cm diam. plastic pots and placed in an outdoor screenhouse. The seedlings were entirely enclosed by no-see-um mesh bags (Balson-Hercules Group, Ltd., Providence, RI, U.S.A.). The mesh bags lined the pots and were supported above the plant by 46 cm high (2.5 cm mesh) wire net cylinders resting on the soil surface in the pots and anchored by two short bamboo stakes. The bags were tied at the top to exclude arthropods yet allow access for sampling.

On 11 July the potted plants were placed in an enclosed screenhouse, the bags opened, and adult *P. rapae* from a laboratory culture released and allowed to oviposit for 24 h. Sixty of the plants were then placed in each of the two cabbage fields, replacing every fifteenth plant in every third row, forming a 30.5 x 24.7 m grid in the plot center. The pots were embedded to just below the soil surface in the plots. On each plant the locations of five well-spaced eggs on the leaf undersides were marked by drawing a circle on the leaf's upper surface with a permanent marker. Any additional eggs were removed. Plants at alternate points of the grid were designated as sham cage plants. On 14 July the lower 15 cm of the above-ground

portion of the mesh bags on these plants was cut away and the upper portion left in place and fastened to the wire netting cylinder using straight pins. The pot rims and soil inside the pots were then covered with field soil to form a continuous surface with the surrounding soil. *P. rapae* on the plants were counted and any newly oviposited eggs or colonizing aphids were removed on 16, 22, and 26 July. By 26 July most larvae had reached fifth instar.

The plants and *P. rapae* were removed from the pots on 26 July and replaced later with new plants (variety "Bravo") in 10 cm diam. pots bearing five *P. rapae* eggs per plant. The five eggs /plantwere obtained by placing the plants in our lab culture's oviposition cage for a few minutes apiece until >5 eggs were laid then removing the excess eggs. The smaller pots were embedded in the soil inside the larger pots that were already in the field such that the soil in the pots formed a continuous surface. The second set of plants was placed in one of the fields on 2 Aug. and in the other on 8 Aug. *P. rapae* were counted and any new *P. rapae* eggs or aphids were removed from the plants every 2–3 d until most of the larvae had reached the fifth instar. For each of the four experiments survivorship curves for the two treatments were compared visually to determine stages at which most mortality occurred. In addition, 95% confidence intervals for total mortality due to treatment effect were constructed using Elston's (1969) method as described by Rosenheim and Hoy (1989).

Monitoring predators on cabbage

Cabbage (variety 'Cheers') was transplanted into 0.2 ha plots at three locations ≥1 km apart on 22–26 May 1995. Sticky traps were placed on 36 plants in a 6 x 6 grid in the center of each plot on 10 July when most of the plants were in precupping stage (13–19 leaves). The outermost trap plants were ≥6.4 m from the plot edges. The traps consisted of two 8.6 cm rings of insect trap coating (Tanglefoot Co., Grand Rapids, MI, U.S.A.) pressed onto the upper and lower surfaces respectively of two opposite frame leaves of each trap plant. A plastic "deli container" (32T, Fabri-Kal Corp., Kalamazoo, MI, U.S.A.) with a hole cut out of the bottom was used to apply the trap coating to the leaf surfaces. The traps were checked every 3–4 d and all predaceous arthropods removed and preserved for identification. Each week the coated leaves were removed and new trap coating applied to leaves of the same or nearby plants. Trapping continued until 14 August when the plants were close to maturity.

Predation in small arenas

Species of arthropod predators that were most abundant in the sticky traps were collected from cabbage fields and surrounding weedy areas both manually and by use of dry pitfall traps. Predators were placed individually into white plastic 7.6 cm high 11.8 cm diam. arenas with clear plastic lids (Fabri-Kal Corp., Kalamazoo, MI, U.S.A.). The containers were lined with filter paper and provided with a moist 2.5 cm length of dental wick. The arenas containing predators were placed in an environmental chamber at 22:17 (L:D) °C, 60% RH, and 15:9 (L:D) h and starved for 24 h before adding either 10 or 20 *P. rapae* eggs or five first instars. Eggs and first instars were used because the exclusion cage experiments indicated that virtually all predation occurred during these stages. The eggs were presented on pieces of parafilm cut from egg sheets used for oviposition in our *P. rapae* laboratory culture. The larvae were transferred shortly after hatching from egg sheets to 3.2 cm cabbage leaf disks which were then placed with the predators. After an additional 24 h in the environmental chamber, remaining eggs and larvae were counted. A minimum of five control arenas identical to the test arenas except without predators were included with each batch of predators tested. No eggs or larvae were damaged or disappeared in any of the control arenas.

Predation on cabbage plants in the laboratory

Three of the four most abundant predator species captured on sticky traps were further tested for predation ability on cabbage plants in the laboratory. Predators were collected by hand or by dry pitfall traps and starved for 24 h as in the small arena assays. Groups of predators were then placed in plastic cylinder cages with screen tops covering small (4–6 leaf) cabbage plants that had been transplanted into 25 cm diam. plastic pots and infested with ten first instar *P. rapae*. A moist 2.5 cm length of dental wick was placed on the surface in each pot. Three control plants with *P. rapae* but no predators were included in each trial. After 24 h at 22 °C, 40% RH, and 16:8 (L:D) h, remaining *P. rapae* larvae were counted. No larvae disappeared from any of the control plants in the three experiments.

Results

Estimation of mortality from arthropod predation

The survivorship curves for the four experiments showed a reduction in survival of the larvae on plants in the sham cages versus those in the predator exclusion cages. In all cases almost all of the difference arose during the egg or first instar. The bulk of the mortality (52–84%) was common to both cage types, apparently arising from factors that acted equally on *P. rapae* in both treatments. The 95% confidence intervals for total mortality attributable to the treatment were 58 ± 22% and 51 ± 21% for the two fields in the July experiments and 79 ± 19% and 71 ± 20% for the same fields in the August experiments.

Monitoring predators on cabbage

The most abundant predatory arthropods identified on the traps were *Lygus* sp. (Hemiptera: Miridae) (almost all *Lygus lineolaris* Palisot de Beauvois) (305 captured), *Stenolophus comma* F. (Coleoptera: Carabidae) (126), *Coleomegilla maculata lengi* Timberlake (Coleoptera: Coccinellidae) (125), and *Phalangium opilio* (L.) (Opiliones: Phalangiidae) (56). Predators that were caught less frequently included

other Coccinellidae (44), Syrphidae (adults) (33), other Carabidae (27), Anthicidae (21), Araneae (9), Vespidae (8), Sphecidae (4), Nabidae (3), Chrysopidae (2), Pompilidae (2), and Cleridae (2).

Predation in small arenas

As shown in *Tables 1* and *2*, all the predator species fed on *P. rapae* to some extent. Because number of prey per predator was limited in the experiment, it is difficult to evaluate relative voracity of those species that tended to consume most of the prey provided. However, the proportion of individuals that fed on at least one prey can be used to compare the propensity of the different species to feed on *P. rapae.*

Predation on cabbage plants in the laboratory

Both *C. m. lengi* and *P. opilio* fed on *P. rapae* first instars on cabbage plants *(Table 3)*, however many larvae escaped predation in spite of the high number of predators per plant. *L. lineolaris* did not prey on *P. rapae* larvae on the plants.

Discussion

The confidence intervals for mortality of *P. rapae* in the predator exclusion experiments, though large, provide convincing evidence of a high level of arthropod predation in the field. Because *P. rapae* larvae rarely leave suitable host plants (Harcourt, 1961; Jones, 1977), the treatment effects are not likely a result of dispersal from the sham cage plants. Although birds were occasionally seen in the plots, they were excluded from all the experimental plants by the wire netting and cloth covers, so could not have been responsible for the observed differences.

Other studies that have evaluated the impact of predators explicitly have concluded that arthropod predators caused the bulk of mortality in immature *P. rapae* (Dempster, 1967; Parker, 1970; Ashby, 1974; Hasui, 1977; Jones, 1987). While predation was significant in our study, mortality attributable to predators excluded by our cages was much less than mortality resulting from factors that were not excluded. These factors could not be identified, but may include weather effects or small predators such as thrips or mites that could penetrate the cage cloth. Predators that were excluded by the cages caused high levels of mortality to the remaining *P. rapae* in the sham cages.

Our sticky traps and laboratory predation experiments identified two species that are probably at least partly responsible for the mortality observed in the exclusion experiments: *C. m. lengi* and *P. opilio*. *L. lineolaris* appears less likely to prey on *P. rapae* in the field, and although *S. comma* was relatively abundant in the sticky traps and fed readily on *P. rapae* eggs and first instars, its ability to forage on cabbage plants must still be evaluated. Currently we are attempting to quantify the contribution of each species to prey mortality by estimating relative densities and predation frequencies in the field.

Table 1. Consumption of *P. rapae* eggs by individual predators in small arenas

Species	No. prey provided	Mean prey consumed	SD	Percent consuming ≥ 1 prey	No. tested
Coleomegilla maculata lengi	10	6.0	4.9	65	20
Stenolophus comma	20	17.0	7.3	85	20
Phalangium opilio adults	20	12.6	8.1	84	43
Phalangium opilio immatures	10	5.6	4.4	80	20
Lygus lineolaris	10	3.3	4.6	40	20

Table 2. Consumption of *P. rapae* first instars by individual predators in small arenas

Species	No. prey provided	Mean prey consumed	SD	Percent consuming ≥ 1 prey	No. tested
Coleomegilla maculata lengi	5	4.3	1.5	96	23
Stenolophus comma	5	4.1	1.7	88	25
Phalangium opilio adults	5	2.9	1.8	88	49
Phalangium opilio immatures	5	4.1	1.6	95	21
Lygus lineolaris	5	0.6	1.0	43	23

Table 3. Consumption of *P. rapae* first instars on potted cabbage plants by groups of predators (10 *P. rapae* placed on each plant initially)

Species	Predators per plant	Mean prey consumed	SD	N
*Coleomegilla maculata leng*i	6	5.70	3.37	10
Phalangium opilio adults	4	2.00	2.00	6
Lygus lineolaris	6	0	0	10

While the sticky traps indicate occurrence of predatory arthropods on the cabbage leaves, a species' occurrence in trap catches does not necessarily imply that it was foraging on the foliage. Many individuals may become entrapped only after inadvertently alighting on the sticky surface. Conversely, a species' absence in the trap catch does not necessarily imply that it does not occur on cabbage foliage. Because species differ in their susceptibility to sticky traps, their relative abundances in trap catches may not correspond to their relative abundances on the foliage. Under our laboratory conditions all species except *L. lineolaris* showed a high propensity to feed on eggs and first instars. Actual impact of a species in the field, however, will depend on many factors, including predator and prey abundance, searching ability on plants, alternative prey, and numerical and functional responses to prey density. Much additional work is necessary to evaluate the relative contribution of each of the predator species to mortality of *P. rapae* in cabbage fields.

Although more work is needed, our initial results suggest that arthropod predators can contribute a great deal to reduction of *P. rapae* in the field. Because these predators are often generalists, they most likely contribute to reduction of other crucifer Lepidoptera as well and have the potential to persist in the field at low pest densities by subsisting on alternative prey (Ehler, 1977; Reichert and Lockley, 1984; Murdoch *et al.*, 1985). In addition, unlike many parasitoids, these predators act primarily on the early stages of the pest, killing it before it reaches the most damaging late larval instars. These desirable qualities, along with evidence of their importance from this and other studies, justify continued efforts to elucidate the effects of arthropod predators on *P. rapae* and other cabbage Lepidoptera.

Acknowledgments

The authors thank Rick Piccioni, Mary Balsley, Bill Wilsey, and Juliet Tang for technical assistance. This work was supported in part by the New York State Integrated Pest Management Program.

References

Ashby, J. W. (1974). A study of arthropod predation of *Pieris rapae* L. using serological and exclusion techniques. *Journal of Applied Ecology* ***11:*** 419–425.

Dempster, J. P. (1967). The control of *Pieris rapae* with DDT. I. The natural mortality of the young stages of *Pieris*. *Journal of Applied Ecology* ***4:*** 485–500.

Ehler, L .E. (1977). Natural enemies of cabbage looper on cotton in the San Joaquin Valley. *Hilgardia* ***45:*** 73–106.

Harcourt, D.G. (1966). Major factors in survival of the immature stages of *Pieris rapae* (L.). *Canadian Entomologist* ***98:*** 653–662.

Hasui, H. (1977). On the seasonal variability of survivorship curves and life tables of *Pieris rapae crucivora* Boisduval (Lepidoptera: Pieridae) *Japanese Journal of Ecology* ***27:*** 75–82.

Jones, R. E. (1977). Search behaviour: a study of three caterpillar species. *Behaviour* ***60:*** 237–259.

Jones, R. E. (1981). The cabbage butterfly, *Pieris rapae* (L.): 'A just sense of how not to fly'. In *The Ecology of Pests: Some Australian Case Histories* (ed. R. L. Kitching and R. E. Jones) pp. 217–228. Melbourne: CSIRO.

Jones, R. E. (1987). Ants, parasitoids, and the cabbage butterfly *Pieris rapae*. *Journal of Animal Ecology* ***56:*** 739–749.

Murdoch, W. W. (1985). Biological control in theory and practice. *The American Naturalist* ***125:*** 344–366.

Parker, F. D. (1970). Seasonal mortality and survival of *Pieris rapae* (L.) (Lepidoptera: Pieridae) in Missouri and the effect of introducing an egg parasite, *Trichogramma evanescens* Westwood. *Annals of the Entomological Society of America* ***63:*** 985–999.

Riechert, S. E. and Lockley, T. (1984). Spiders as biological control agents. *Annual Review of Entomology* ***29:*** 299–320.

Talekar, N. S. and Shelton, A. M. (1993). Biology, ecology, and management of the diamondback moth. *Annual Review of Entomology* ***38:*** 275–301.

The status of diamondback moth and its natural enemies in the Forto Novo and Cotonou areas in Benin

D. Bordat[1] and A. E. Goudegnon[2]

[1]*Centre de Coopération Internationale en Recherche Agronomique pour le Développement, Département des productions Fruitières, Légumières et Horticoles (CIRAD-FLHOR), Laboratoire d'Entomologie, BP 5035, 34032 Montpellier Cedex 1, France*

[2]*Faculté Agronomique des Sciences et Technique, Université Nationale du Bénin (FAST-UNB), Laboratoire de Zoologie, Cotonou, Bénin.*

Abstract

Plutella xylostella (L.) is year round the major pest in cabbage fields in the coastal zones of Benin, particulary in the Cotonou periurban area and in the Porto Novo lagoon. In these two areas, only 40 km apart, *P. xylostella* seems to have different population dynamics and parasitoid populations. In Cotonou, the highest population level of *P. xylostella* are observed in March and in December. The larval parasitoid *Cotesia plutellae* (Kurdjumov) is present all year round but its populations increase in May, October and December. In Porto Novo, the highest population level of *P. xylostella* is observed in September and *C. plutellae* is not present at all. In the both areas, some *Euplectrus laphygmae* Ferrière are found on *P. xylostella* larvae.

Key words: *Plutella xylostella, Cotesia plutellae*, population dynamics, Benin

Introduction

Among the pests in cabbage fields in Benin, the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), is the most significant (Bordat & Goudegnon, 1991). It is found everywhere but particulary in the coastal zones where the humidity (80%) and the high temperature (28 °C) are present all year round. To control DBM, farmers use pesticides but populations have become resistant to deltametrin, the most common pesticide used in Benin against this pest (Goudegnon & Bordat, 1992). Before beginning Integrated Pest Management programs, it is first necessary to know the population dynamics of this pest. In this study, samples were taken over a one year period from each of two localities, the periurban area of Cotonou and the Porto Novo lagoon.

Materials and Methods

Samples (entire cabbage) were harvested from vegetable growers fields. The variety KK Cross was grown by the method usually employed in Benin: patches of 3 rows of cabbage, with 50 cm between rows and 40 cm between each cabbage.

The survey was done twice per month and for one year (1995). Every two weeks, a random selection of 20 cabbages was taken from each of the two localities. The cabbages were dissected, larvae and pupae of DBM were recuperated and reared. All parasitoids emerging from DBM were counted and identified in the laboratory of Taxonomy and Faunistic of CIRAD in Montpellier. The number of adults DBM were also counted.

Results

In the periurban area of Cotonou, DBM populations increased sharply in March and then declined until June. Populations began to rise again in October and were still high in December *(Figure 1)*. The population curve of the main parasitoid, *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae), had the same shape as DBM albeit with lower overall levels. Furthermore, the increases and decreases were later, i.e. a rise was seen in May, October and December *(Figure 1)*. Some individuals of *Euplectrus laphygmae* Ferrière, (Hymenoptera: Eulophidae), were found in February, March, May, August and December *(Figure 1)*.

Parasitism of DBM larvae by *C. plutellae* was found to be efficient (59%) despite a level of hyperparasitism near 20%. Hyperparasitism was principally due to *Trichomalopsis* af. *lasiocampae* (Grahman), (Hymenoptera: Pteromalidae) (Grahman, 1969), 13%, and *Aphanogmus reticulatus* (Fouts), (Hymenoptera: Ceraphronidae) (Dessart, 1971), 6%, *(Figure 2)*.

In the lagoon of Porto Novo, DBM populations increased slowly in March and then decreased until June. Population levels rose again sharply in September and then decreased until December *(Figure 3)*. No adults of *C. plutellae* were found but some *E. laphygmae* individuals were found in February, May and September *(Figure 3)*.

Discussion

Although Cotonou and the lagoon of Porto Novo are found only 40 km apart and share a similar climate, population dynamics of DBM were found to be quire different in each of the two localities in Benin. There

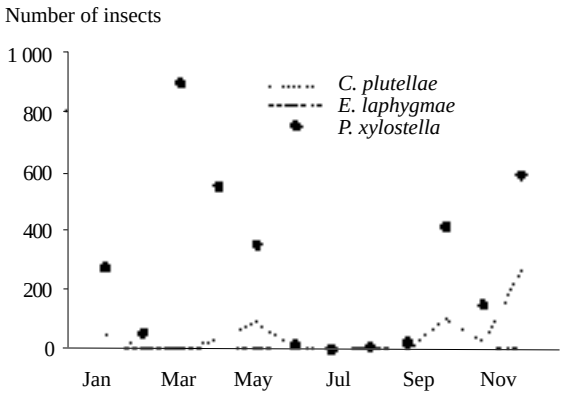


Figure 1. Distribution of DBM and associated parasitoid populations in Cotonou cabbage fields (40 cabbages sampled per observation date

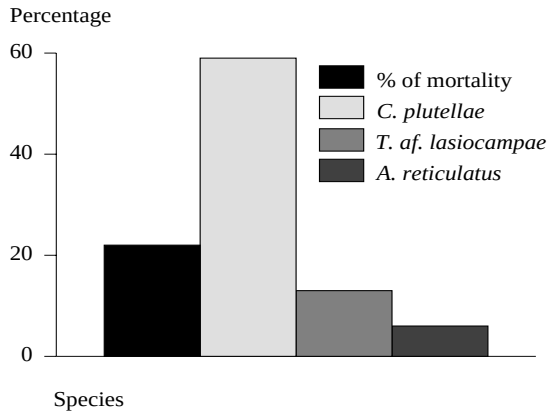


Figure 2. Percentage of parasitoids (C. plutellae) and hyperparasitoids on DBM populations in Cotonou cabbage fields

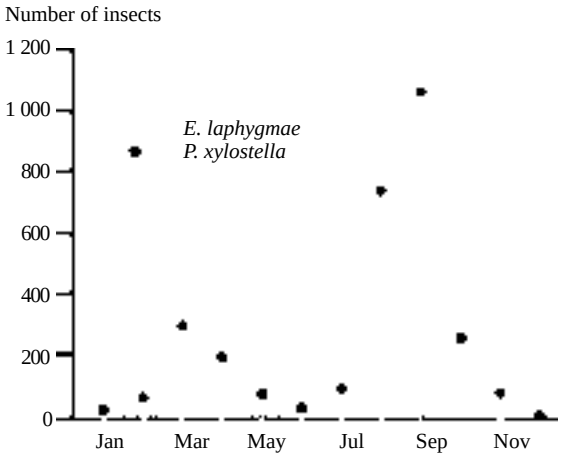


Figure 3. Distribution of DBM and associated parasitoid populations in Porto Novo cabbage fields (40 cabbages sampled per observation date).

are several possibilities to explain this phenomenon. First, the dissimilarities in biotope: in Cotonou, large areas were cultivated with cabbage without tree cover, whereas in lagoon of Porto Novo, cabbage was grown under coconut and banana trees. Secondly, in the lagoon and until recently, the main culture was *Amaranthus* and cabbage has only been a year round crop for a few years. So it is possible that the DBM populations are not yet well established. It is also possible that for this same reason *C. plutellae* populations are not yet established.

In Cotonou and in Porto Novo, the small percentage of parasitism of DBM larvae by *E. laphygmae* is probably due to the fact that DBM larvae is not the ideal host for this species. Its females probably prefer to lay their eggs on the larvae of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) which is also present on cabbage leaves and is the preferential host of this species.

The decrease in DBM populations in the two localities is likeky due to the strong rains which wash the eggs of the pest from the cabbage leaves. Consequently, the number of larvae present on leaves will be lower.

It must be noted that in Cotonou, high population levels of *Lipaphis erysimi* (Kaltenbach) Homoptera: Aphididae, damage the cabbage from July to September, making it unfit for DBM larval consumption.

The results outlined in this study were from samples taken over only a one year period. These results must be confirmed over the next two years. This ongoing study has continued and, so far, the results appear to be the same, as those reported here, for the both areas.

Acknowledgement

We express appreciation to all vegetable growers of the periurban area of Cotonou and of the lagoon of Porto Novo for giving us permission to sample in their fields, without that this study would not have been possible. We also thank Dr. G. Delvare (CIRAD) for the identification of the species of parasitoids and hyperparasitoids, and Dr. C. Kaye (University of Florida) for critical reviews of this manuscript.

References

Bordat, D. & Goudegnon, E. A., (1991). Catalogue des principaux ravageurs des cultures maraîchères au Bénin. *Rapport CIRAD-FLHOR*, Montpellier, France. pp. 1–38.

Dessart P., (1971). Transferts génétiques de quelques Ceraphronidae (Hym. Ceraphronidea), *Bull. Ann. Soc. R. Ent. Belg.*,107, 99–100.

Goudegnon, E. A. & Bordat D., (1992). Comparaison de l'efficacité d'une formulation à base de *Bacillus thuringiensis* et d'un insecticide chimique pour lutter contre *Plutella xylostella* (L.) au Bénin. Sensibilité de six espèces de Lépidoptères ravageurs des cultures légumières à cette formulation. *Rapport CIRAD-FLHOR*, Montpellier, France. pp. 1–19.

Graham (De Vere) M. W. R., (1969). The Pteromalidae of North Western Europe (Hym; Chalcidoidea). *Bulletin of the British Museum*, Entomology, London 1969, pp. 25–36.

Influence of HD1 on the developmental stages of *Diadegma* sp. parasitoid of diamondback moth

R. G. Monerrat[1] and D. Bordat[2]

[1]*Empresa Brasileira de Pesquisa Agropecuária , Centro National de Pesquisas em Recursos Geneticos e Biotecnologia (EMBRAPA-CENARGEN), Parque Rural, Caixa Postal 02372, Brasilia DF, Brazil*

[2]*Centre de Coopération Internationale en Recherche Agronomique pour le Développement, Département des Rroductions Fruitières, Légumières et Horticoles (CIRAD-FLHOR), Laboratoire d'Entomologie, BP 5035, 34032 Montpellier Cedex 1, France*

Abstract

Laboratory experiments have shown that HD1 strain of *Bacillus thuringiensis* Berliner has adverse effects on populations of *Diadegma* sp. Young larvae of *Plutella xylostella* (L.), the diamondback moth (DBM) were killed by *B. thuringiensis* when the larvae were parasitized by *Diadegma* for only a short time (less than two days). The larvae parasitized for more than two days before adding the bacterium, however, decrease their foliar consumption (35% less) and therefore, consumed fewer spores of *B. thuringiensis.* The low mortality of the DBM allows the parasitoid to finish its development. The presence of HD1 does not effect the F1 generation of *Diadegma* sp. There are no significant differences in the time of the larval stage and pupal development, sex-ratio, longevity of adults and percentage of parasitism between adults from HD1 infected or non-infected larvae.

Key words: *Diadegma* sp., *Bacillus thuringiensis*, interactions.

Introduction

The diamondback moth (DBM), *Plutella xylostella* (L.) Lepidoptera: Yponomeutidae, is presently the most important pest on Brassicae (Talekar & Shelton, 1993). Originally from the Western European mediterranean area, this species has spread to all five continents following the development of cabbage crops (C.I.E., 1967). The cost of controlling this pest, which can produce near 20 generations per year in tropical climates, can be estimated as between 30 and 50% of the cost of cabbage production (Lim, 1986). The cost of controlling the DBM was estimated at about 1 billion US$ (Talekar *et al*., 1990). Because of the rapid development of pesticide resistance in the DBM populations, research is moving towards using natural enemies, principally parasitoids and entomopathogenous organisms. Of the entomophages of the DBM, the genus *Diadegma* Hymenoptera Ichneumonidae, has many species that are particulary efficient natural enemies of the DBM (Talekar *et al*., 1990). Despite the appearance of the DBM populations resistant to *B. thuringiensis*, sprays of this bacterium are often used in ecosystems where *Diadegma* populations are present (Monnerat, 1995). This study is aimed at evaluating the effects of *B. thuringiensis* strains on the developmental stages of one *Diadegma* species.

Materials and Methods

Cultures of the DBM originated from Réunion island. Rearing conditions were 25°C; 75% relative humidity; a photoperiod of 16:8 (L:D) h. Adults were introduced into a transparent cage used as an oviposition container. Females laid their eggs directly on a cabbage (about 10 leaves) grown in a pot. Each day, the cabbage was removed from the cage and replaced with a new one. Larvae completed their development on the same plant. Two days before larvae reached pupation they were placed in a plastic box with the top covered with fine mesh and offered fresh cabbage leaves. Pupae were collected three times per week. Adults were introduced into the oviposition container as they emerged from pupae. Under our rearing conditions, complete development took about 20 days.

Cultures of *Diadegma* also originated from Réunion island. Rearing conditions were the same as for the DBM. Adults were introduced into a cubic oviposition container. The top was covered with fine mesh for aeration. Adults were fed with a few drops of honey and water was provided on moistened cotton. The DBM larvae (10 larvae per female) were introduced into the cage on a cabbage leaf. After 24 h, larvae were remove from the cage and reared until cocoon formation. Three times per week, cocoons were collected and newly emerged adults were introduced into the oviposition container. Under our rearing conditions, development of *Diadegma* took about 15 days. Several adults of this *Diadegma* species were deposited into the collection of the Faunistic and Taxonomy Laboratory of Montpellier CIRAD Center to provide samples that will aid in developing a system of identification (Fitton *et al*., 1991; Noyes, 1994).

In this study, we were not able obtain a purified HD1 strain. We therefore used a commercial formulation (Biobit®) containing it. This formulation gave good results in Brazil (Monnerat, 1995).

Influence on the larval stage

Third instars of the DBM were removed from the rearing boxes and ramdomly separated into four groups. Two groups were used as control (non-parasitized larvae) and the other two were exposed to *Diadegma* females. One group of larvae was parasitized for one day and another group of larvae for three days. In each instance, some non-parasitized larvae were tested with the commercial product.

The method used was near that currently used for resistance tests (Tabashnik *et al*., 1990) with some modifications. Cabbage leaf disks were replaced by young leaves, because they did not dry on the edges like disks and because they have less wax they allow better adherence of the HD1 solution to the dried cabbage leaves (Monnerat, 1995).

After their death, parasitized larvae were dissected to confirm parasitism. Mortality was evaluated after 48 h and after five days. After 48 h the treated cabbage leaves were replaced by non-treated leaves. The results were recorded for five replicates, data were submitted to Probit analysis and were expressed as LC 50.

Influence on the F1 biology

Parasitoids used in this test came from the DBM larvae infected with HD1 at the LC 50 (15 µg/ml) obtained in previous tests. Three hundred larvae were used to obtain the five pairs of *Diadegma* necessary for each replicate. The adults were isolated after emerging from the cocoons. Five newly emerged pairs of *Diadegma* were placed in a cubic transparent plastic cage, as was used for the rearing program. The following day, 50 second instar DBM were put on a cabbage leaf, were introduced into the cage and were allowed to remain with the females for 24 h. They were then removed from the cage and placed in a rearing chamber to allow the development of the parasitoid. When adults emerged from the cocoons, they were counted and sexed.

This test was repeated three times. The data obtained about the productivity, longevity of adults, percentage of parasitism, sex-ratio of the F1, times to emergence of cocoons and adults were submitted to variance analysis by Newman-Keuls test.

Results and Discussion

Influence on the larval stage

The DBM larvae parasitized by *Diadegma* for one day and then infected by HD1 have an LC 50 near non-parasitized larvae, 14.588 and 13.282, respectively, after 48 h, and 2.449 and 2.141 after five days *(Table 1)*. When larvae were parasitized for three days, LC 50 was different from non-parasitized larvae, 25.420 and 12.488, respectively, after 48 h, 4.823 and 2.277 after five days *(Table 1)*.

This difference in sensitivity can be explained because larvae parasitized for 24 h have not been weakened by the parasitoid and consume the same quantity of cabbage leaf as non-parasitized larvae. Three days after *Diadegma* laying, however, the 2nd instar parasitoid began to weaken the host larvae which decrease their foliar consumption by about 35% (Monnerat, 1995), and therefore absorb less HD1. We conclude that, in a natural population of the DBM, it is possible that only young parasitized larvae are likely to be destroyed by sprays of HD1, if *Diadegma* is also present.

Influence on the F1 biology

The duration of *Diadegma* larvae development from the DBM larvae infected with HD1 was similar to that of non-infected larvae. Cocoons appeared between the

Table 1. Mean lethal concentration (g/ml) for the DBM larvae parasitized by *Diadegma* sp. and infected by HD1

DBM	Nb larvae	Slope	LC 50 (48 h)	Slope	LC 50 (5 d)
Parasitized for 1 day	150	1.29	14.588 (10.527–19.768)	1.71	2.449 (1.933–3.103)
Non-parasitized	150	1.54	13.282 (9.955–17.773)	1.32	1.141 (1.418–2.952)
Parasitized for 3 days	150	1.80	25.420 (19.993–32.417)	2.15	4.823 (4.092–5.685)
Non-parasitized	150	1.73	12.488 (10.107–15.569)	1.77	2.277 (1.798–2.848)

Results submitted to Probit analysis

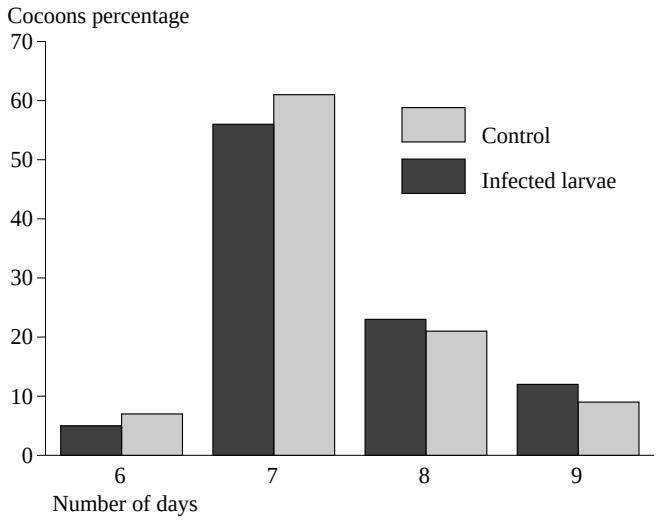


Figure 1. Duration of pre-imaginal development and percentage of Diadegma cocoons from DBM larvae infected and not infected by HD1

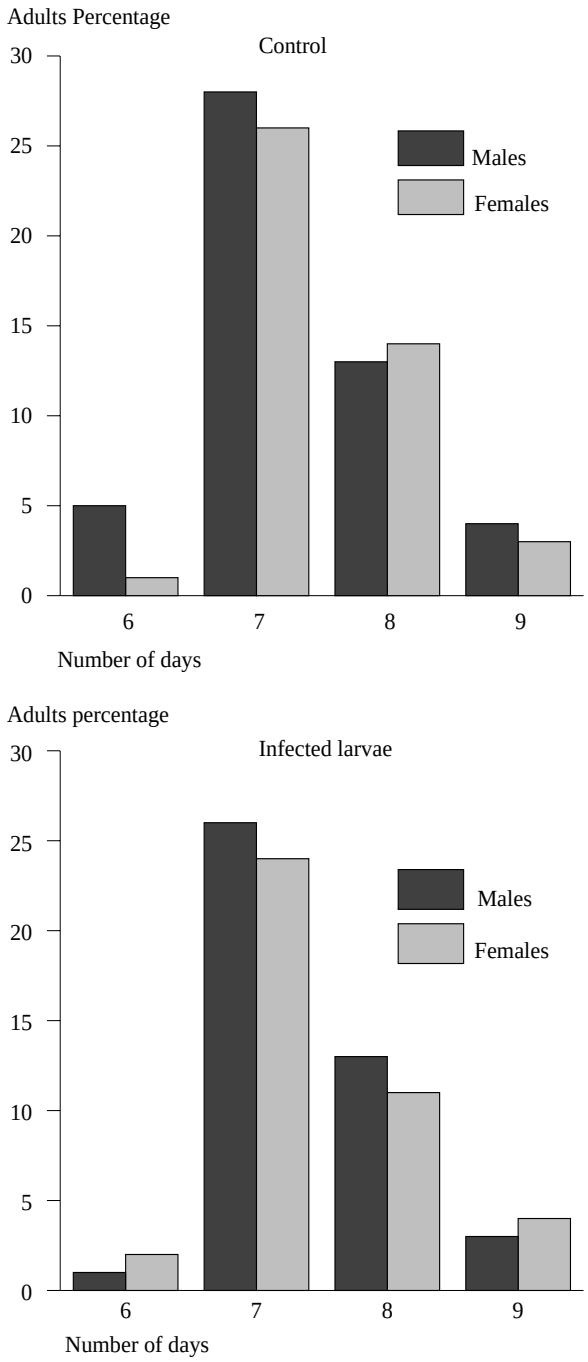


Figure 2. Duration of pupal development and sex-ratio of Diadegma sp. from larvae infected and not infected by HD1

Table 2. Percentage of parasitism and longevity of *Diadegma* adults obtained from DBM larvae infected and not infected by HD1

	Control	Infected larvae
Male longevity (days)	17a	13a
Female longevity (days)	9b	7b
Percentage parasitism	72c	76c

Values followed by similar alphabets across a row are not significantly different ($p<0.05$; Newman–Keuls test)

6th and 9th day after laying with the maximum number appearing on the 7th day *(Figure 1)*.

The duration of the pupal stage and sex-ratio of adults obtained were not significantly different *(Figure 2)*. The adults emerged between the 6th and 9th day after cocoon formation with maximum number emerging on the 7th day *(Figure 2)*

Males live two times longer than females the same as the controls, and the adults obtained parasitized the DBM larvae at the same percentage, about 70% *(Table 2)*.

The presence of HD1 in DBM larvae did not disturb the biological cycle of the *Diadegma* parasitoid and toxins produced had no effect against the parasitoid when present in the infected DBM larvae. It is therefore possible to combine HD1 and *Diadegma* sp. in IPM programs against the DBM.

But it will be very important to confirm these results in a natural conditions, and in laboratory on other species of *Diadegma* and on other parasitoid species such as Braconidae, *Cotesia plutellae* (Kurdjumov) a very efficient species regularly present in sizeable numbers in the DBM populations and currently present on all five continents.

Acknowledgement

We express appreciation to C. Pagès and C. Tertois (CIRAD-FLHOR) for technical assistance, and Dr. C. Kaye (University of Florida) for critical reviews of this manuscript.

References

C.I.E. (1967). *Plutella xylostella* (L.) Lep. Plutellidae, Diamond-back Moth. *Distribution Maps of Pests*, 1–3.

Fitton, M. G., Walker, A. K. & Lasalle, J. (1991). Nomenclature of *Plutella* parasitoids. *IOBC Newsletter*, 3–5.

Lim, G. S. (1986). Biological control of Diamondback moth. *Proceedings of the first International Workshop*, 1985, Tainan, Taïwan, pp. 159–171.

Monnerat, R. G. (1995). Interrelations entre la Teigne des Crucifères *Plutella xylostella* (L.) (Lep: Yponomeutidae), son parasitoïde *Diadegma* sp. (Hym: Ichneumonidae) et la bactérie entomopathogène *Bacillus thuringiensis* Berliner. *Thèse de Doctorat en Sciences Agronomiques*, ENSA Montpellier, France, pp. 1–162.

Noyes, J. S. (1994). The reliability of published host-parasitoid records: a taxonomist's view. *Norwegian Journal of Agricultural Sciences*, 16, 59–69.

Tabashnik, B. E., Cushing, N. L., Finson, N. & Johnson, M. W. (1990). Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lep: Plutellidae). *Journal of Economic Entomology*, ***83:*** 1 671–1 676.

Talekar, N. S. & Shelton, A. M. (1993). Biology, ecology and management of the diamondback moth. *Annual Review of Entomology*, ***38:*** 275–301.

Talekar, N. S., Yang, J. C. & Lee, S. T. (1990). Introduction of *Diadegma semiclausum* to control diamondback moth in Taiwan. *Proceedings of the Second International Workshop*, Tainan, Taiwan, 1990, pp. 263–270.

Monitoring of insecticide resistance in diamondback moth based on cholinesterase genotype

Masayasu Kato[1], Thomas M. Brown[2], Tadashi Miyata

Laboratory of Applied Entomology, School of Agricultural Sciences, Nagoya University, Chikusa, Nagoya 464-01, JAPAN

Present Address: [1]Ciba-Geigy Japan Limited, 4-1, Hamamatsu-cho, 2-chome Minatoku, Tokyo 105, JAPAN, [2]Department of Entomology, Clemson University, Clemson, South Carolina 29634-0365, USA.

Abstract

Possibility of monitoring insecticide resistance in the diamondback moth by analyzing the insensitivity of cholinesterase (ChE) which is resistant to organophosphates and carbamates was investigated. Four kinds of ChE genotypes were found and inherited by incomplete dominance factors. Against propoxur and monocrotophos, each ChE genotype showed LD50 in proportion to the values of I_{50}. Difference of I_{50} values in four kinds of ChE genotype to various ChE inhibitors was highest for methomyl(30 times), and very low for carbofuran and metolcarb(2–3 times). There is no sensitive ChE to both insecticides or very few in this strain. We also investigated other insecticide resistant strains and populations collected in the fields. IGR resistant and susceptible strains collected in Thailand and representative Japanese susceptible strain possessed only sensitive ChE. In two diamondback moth populations collected in Japan in 1992 and 1993, there were very few sensitive ChE, but more insensitive ChE. Existence of various types of ChE in the diamondback moth collected in fields means a potential to develop insecticide resistance by insecticide selection. As insecticide sensitivity changes from a low to 30 times according to ChE genotypes, it was considered that ratios of ChE genotypes were quickly affected by insecticide selections.

Key words: Diamondback moth, AChE, resistance, monitoring

Introduction

Cruciferous crops are cultivated around the world from the temperate to the tropical zones especially in Asia, where these are very important crops. Diamondback moth *(Plutella xylostella)* is among the most destructive insect pests for these crops. Since the development of resistance to DDT in Indonesia (Ankersmit, 1953), diamondback moth (DBM) has developed resistance to various insecticides (Sudderruddin and Kok, 1978: Sun *et al.*, 1978:Sinchaisri *et al.*, 1980: Tang *et al.*, 1988). The development of resistance to *Bacillus thuringiensis* and IGR which were introduced in recent years, have been reported (Perng *et al.*, 1988: Tabashnik, 1990).

In combinations of chlorinated hydrocarbons with synthetic pyrethroids and organophosphates with carbamates, cross resistance was often reported. However, in other combinations it was hardly reported. Combinations of the same class of insecticides do not always show cross resistance (Sasaki, 1982: Noppun *et al.*, 1987a).

DBM is well-known for the instability of its insecticide resistance. Generally, in DBM the high insecticides resistance level soon declines to the low level under no insecticides pressure (Miyata *et al.*, 1986; Chen and Sun, 1986). Hence, it is possible that insecticides which have not been used for a long time in the field due to the insecticide resistance can become very effective again (Saito *et al.*, 1990). Moores and Devonshire (1984) reported a rapid technique to check the insensitivity of ChE (cholinesterase) in single houseflies to organophosphates and carbamates. Subsequently, *Heliothis virescens* (Brown and Bryson, 1992), mosquito (Ffrench-constant and Bonning, 1989) and *Bemisia tabaci* (Bryne and Devonshire, 1993) were investigated by the same method.

We report here the possibility of insecticide resistance monitoring based on the insensitivity of ChE in single insects.

Materials and Methods

In this experiment six strains of DBM were investigated. The origin of these strains is shown in *Table 1*. The insects were cultured at 25 °C (16L:8D) without exposure to any insecticides. The rearing method was slightly modified from the report of Noppun *et al.* (1983).

The following insecticides of a technical or analytical grade were obtained from the respective manufacturers: propoxur (98.2%), carbofuran (99.0%), isoprocarb (99%), monocrotophos (99.6%), methomyl (91.8%), metolcarb (97.4%), phenthoateoxon (99%).

Enzyme preparation. Individual heads from frozen DBM adults were used as sources of acetylcholinesterase. They were homogenized in 0.1 M MOPS, pH 7.5 buffer by ULTRA DISRUPTOR UV-200 (Tomy Co.) using output strength 1 for 3 seconds twice, in polyethylene tube cooled with ice. Supernatant following 2.0 min. of centrifugation at 16000x g (4 °C) was used as the enzyme source.

Table 1. The origin of the diamondback moth used

Strain	Place*	Time**	Status
OSS	Osaka	1969	Susceptible strain (Noppun *et al.*, 1984)
OKR-R	Okinawa	1980	Phenthoate resistance(Noppun *et al.*, 1986). Regularly selection bu phenthate.
BK-R	Bang Khae	1987	Chlorfluazuron resistance (Fahmy *et al.*, 1991) Regularly selection by chlorfluazuron.
BK-S	Bang Khae	1987	Chlorfluazuron susceptible (Fahmy *et al.*, 1991) Regularly selection for susceptibility to chlorfluazuron
Okinawa	Okinawa	1992	3rd generation after collecting in the field.
Tukuba	Ibaragi	1993	3rd generation after collecting in the field.

*Place of collection
**Year of collection

Enzyme assay. ChE activity was monitored by Micro Plate Reader (Bio Rad Co.) based on the procedure of Ellman *et al.* (1961) by using a 405 nm filter. For routine assays, 0.5 x 10^{-3}M ATCh, 0.4 x 10^{-3}M, 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) and 0.7 x 10^{-3}M sodium bicarbonate were used. The assay was done at 25 °C .

Grouping of single adults by ChE genotypes. Individual heads was homogenized in 120 µl buffer solution respectively, and enzyme solution was prepared as described above. The 20 µl enzyme solution was added to 3 wells of 96-well plates respectively. In the first well was added 100 µl reaction mixture for the enzyme assay (100 µl acetone was added to 15 ml the reaction mixture containing ATCh, DTNB and sodium bicarbonate), in the second was added 100 µl reaction mixture containing monocrotophos acetone solution, and in the third one was added 100 µl reaction mixture containing propoxur acetone solution. At the reaction, monocrotophos and propoxur were 3.5 x 10^{-3}M and 7 x 10^{-4}M, respectively. After 0 and 60 minutes ChE activities were monitored. The rate of absorbance changes of wells inhibitor added to the control well during 60 minutes were displayed by a scatterplot.

Inhibition test. The I_{50}s by various inhibitors were determined by pre-inhibition method. Inhibitor acetone solutions were mixed with the buffer, and used as inhibition solution. To the control was added only acetone to the buffer. At both the inhibition and the enzyme assay, the concentration of acetone in the inhibition solution and the reaction mixture was prepared at 0.56 %. Three replicates were used at each inhibitor concentration. By adding the inhibition solution to 10 µl enzyme solution in 96-well plate the inhibition was started. Thirty minutes later, by adding 200 µl reaction mixture solution for enzyme assay the inhibition was stopped and monitored ChE activity. Data were analyzed by probit analysis (Finney, 1971).

Toxicity test. The LD_{50}s of the different strains were determined by topical application using a microapplicator (Kiya Seisakusho Co. Ltd., Tokyo). Third instar larvae were anesthetized with CO_2 and treated individually with a Hamilton microsyringe 0.54 µl of insecticide acetone solution to the thoracic dorsum. Treated insects were caged on cabbage leaves in plastic cups at 25 °C and at a photoperiod of 16L8D. We used at least 4 doses between 0 and 100% mortality. Three replicates with 10 larvae per test were used at each dose level. The control larvae were treated with acetone only. Data were analyzed by probit analysis (Finney, 1971).

Back-cross. Pupae were put in glass tubes which were 1 cm diameter and 7.5 cm height, and the tops were covered with cloth. Newly-emerged adults were identified on the distinction of sex with naked eye, and each pair of male and female was moved to plastic cups which were 10.3 cm diameter and 9.7 cm height. In the plastic cups there were cotton moistened with 5% honey dew and cabbage leaf for oviposition. A few days later the oviposition was checked and the pairs were preserved in –80 °C freezer till ChE genotype were identified. After ChE genotypes of the parent were identified, the eggs from the same combination of the parent ChE genotype were cultured in the same case, and the group which would have homozygous ChE gene were made. Ten pairs of the same group were mated and the 100 progenies were identified on ChE genotype. Secondly, every heterozygous combination were pair-mated (5 pairs for each combination) and ChE genotypes were identified. Using these progenies, only 3 kinds of combinations and 5 pairs in each combination were back-crossed, and ChE genotypes of the next 50 progenies in each combination were identified (the reverse combination of sex on ChE genotype was distinguished).

Results

Preliminary experiments estimated that OKR-R strain would have 4 kinds of ChE genes (*A*, *B*, *C* and *D*). The progenies from the parents which belonged to the same cluster in the scatterplot belonged to the same cluster with the parents *(Fig.1)*. The progenies from the parents which belonged to the different clusters each other belong to the cluster in the middle of the parents *(Fig.2)*. In the back-cross experiment 3 kinds of combinations, ♂D x ♀AD, ♂AD x ♀D and ♂AD x ♀A were mated in 5 pairs, and 50 progenies were identified of ChE insensitivity *(Table 2)*. The χ^2 test, the ratio of their ChE types were not different significantly with the expected value and confirmed that they were inherited as Mendelian inheritance and incomplete dominance.

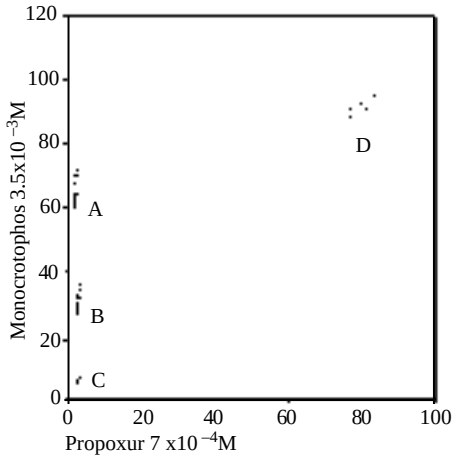


Figure 1. Bivariate plot of mean percentage activity remaining during inhibition of ChE by monocrotophos and propoxur for four ChE which is considered homogenous from OKR strain

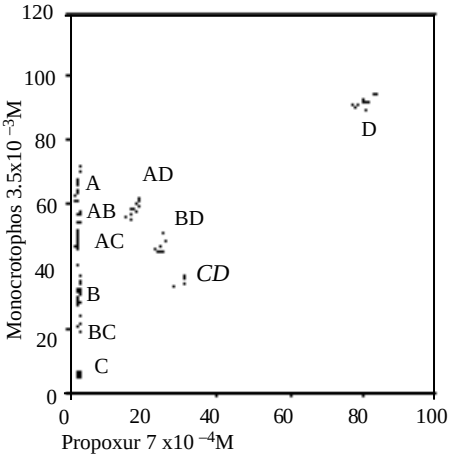


Figure 2. Bivariate plot of mean percetange activity remaining during inhibition of ChE by monocrotophos and propoxur for 10 ChE which is considered homogenous and heterogenous from OKR strain

Table 2. ChE phenotypes of progenies from backcross. In each combination 5 pairs were pair mated, and ChE fenotype of 50 progenies were investigated.

	No. of progenies with each ChE phenotype					
Parent	♂Dx♀AD		♂ADx♀D		♂ADx♀A	
Progeny	D	A	D	AD	A	AD
	25	25	28	22	28	22

Table 3 showed LD_{50} of third instar larvae to propoxur and monocrotophos used for the bivariate plot. *C* type was the most susceptible to monocrotophos and about 10 times as susceptible as others. *C* type was the most susceptible to propoxur too, but *A* type showed also the same level of susceptibility. *D* type was about 10 times as resistant as *C* type to propoxur.

Except carbofuran, *C* type ChE was inhibited most strongly by several insecticides *in vitro (Table 4)*. Carbofuran inhibited *A* type more than *C* type by a narrow margin. Then *C* type ChE was used as a standard, as follows. *A* type ChE was 17, 10 and 14 times as insensitive as *C* type to monocrotophos, methomyl and DTP, respectively. However, *A* type ChE was just 1.2 times as insensitive as *C* type to propoxur and 0.7 times to carbofuran. Thus, *A* type ChE was more sensitive than *C* type ChE to carbofuran. *B* type ChE was 12 and 10 times as insensitive as *C* type to monocrotophos and DTP, and slightly insensitive to other insecticides. *D* type ChE was 30, 11 and 8.3 times as insensitive as *C* type ChE to methomyl, monocrotophos and isoprocarb, respectively, and about 5 times to phenthoate, propoxur and DTP. As *C* type was the most sensitive to almost all insecticides of the 4 types, it was considered that *C* type ChE was a wild type.

Bivariate plots of other DBM strains were given in *Fig.3*. OSS strain, which is a representative susceptible strain, had sensitive *C* type and *BC* type ChE. Resistant (BKR-R) and susceptible (BKR-S) strains to chrolufluazuron which were collected in Thailand and afterward cultured in the laboratory and the former strain was selected in the laboratory and the latter strain was not (Adel *et al*, 1991), had almost only sensitive *C* type ChE. Tukuba strain was gathered in Japan in 1993 and kept frozen. The bivariate plot showed the characteristic of the wild DBM in the area at that time. Tukuba strain had sensitive *C* type ChE a little, and almost were insensitive ones. In Okinawa strain there were no sensitive *C* type ChE, the degree of the sensitivity reduction was stronger than Tukuba strain.

Discussion

From the mating experiment, 4 kinds of ChE genes were found in OKR-R strain, and they were inherited by incomplete dominance *(Table 2)*. We made 4 strains which had homogenious ChE genes.

The order of LD_{50} values of DBM of different ChE genotypes to propoxur and monocrotophos were *D>A>B>C* and *D>B>A=C* ,respectively *(Table 3)*. The DBM which has *C* type ChE was sensitive to the both compounds. Though the *A* type was ten times insensitive to propoxur than *C* type, it showed the same sensitivity to monocrotophos as the *C* type.

In *Heliothis virescens* and *Musca domestica*, relationships of negatively correlated cross resistance dependent on the ChE insensitivity to insecticides were found (Brown and Bryson, 1992: Yamamoto *et al.*, 1993). The four ChEs founded in OKR-R strain did not show such relationships *(Table 4)*. However, all the four ChEs were sensitve to metolcarb and carbofuran, and their sensitivity to the insecticides were almost same. There were not genes to develop insecticide resistance to the both insecticeds by insensitive ChE, or they were very little in OKR-R strain.

In other reports which investigated insensitivity of ChE in single insects (Moores and Devonshire,1984: Brown and Bryson,1992: ffrench-constant and Bonning,1989: Byrne and Devonshire,1993), every insect had a little each of ChE types, and the each strain

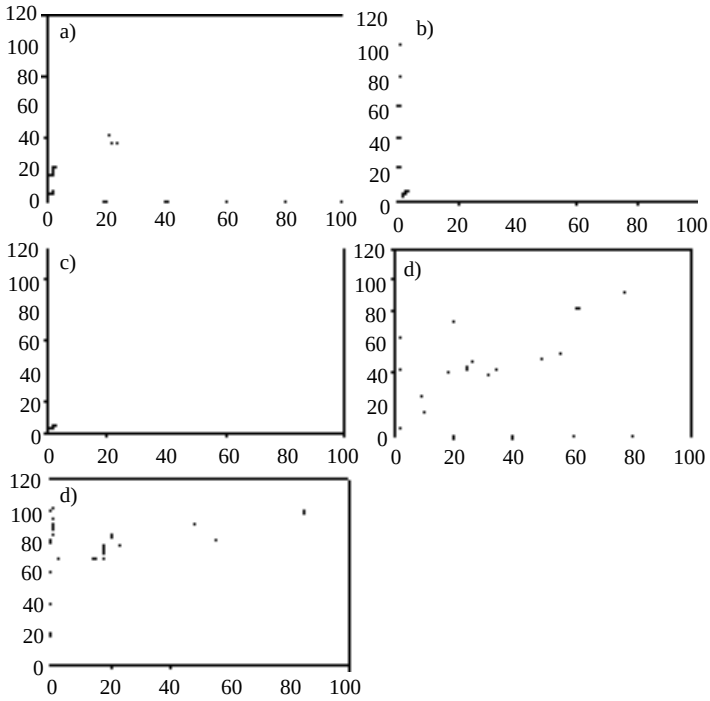


Figure 3. Bivariate plot of mean percentage activity remaining during inhibition of ChE by monocrotophos 3.5 x 10^{-3}M and propoxur 7 x 10^{-4}M. a):OSS strain b): strain c): BKS strain d): Tukuba strain e): Okinawa strain

Table 3. Susceptibility of larvae diamondback moth at 24 hr after topical application

	Insecticide			
	Monocrotophos		Propoxur	
ChE fenotype	LD50(µg/g)	SRE[a]	LD50(µg/g)	SRE
A	34200	2.85	5420	1.93
B	29800	2.42	7960	1.70
C	3730	2.47	3660	1.64
D	45100	1.86	35600	1.56

a)Slope of regression equation.

Table 4. *In vitro* inhibition of DBM ChE by various inhibitors

	10-6xIN50(M)			
	ChE fenotype			
Inhibitor	A	B	C	D
Propoxur	9.23	21.6	7.38	34.7
Monocrotophos	386	268	22.9	262
Phenthoateoxon	4.48	2.72	0.609	3.52
Methomyl	4.11	2.60	0.411	12.2
Isoprocarb	4.57	5.55	1.56	13.0
DTP	35.1	26.5	2.56	12.5
Metolcarb	50.6	54.8	21.9	54.2
Carbofuran	0.131	0.209	0.188	0.498

had almost one type of ChE. The OSS strain which is susceptible strain had just two types of ChE *(Fig. 3)*, but OKR-R strain selected by phenthoate had four types of ChE *(Fig. 2)*. It is interesting that 2 insensitive ChE types (*A* type and *D* type ChE) was found and also found to exist simultaneously in OKR-R strain. Moreover, each type of ChE was different in insensitivity spectrum *(Table 4)*. Tukuba strain and Okinawa strain which were collected in the fields was found to contain many of ChE types *(Fig. 3)*. Tukuba strain had a little sensitive ChE types, but Okinawa strain did not have sensitive ChE types. Both BK-R and BK-S strains were occupied by *C* type ChE *(Fig. 3)*. Although they were collected in recent years, they were different from Japanese strain collected in 1992–1993, occupied by a single sensitive ChE. In

Thailand organophospahtes and carbamates which are inhibitors of AChE have not used for a long years (Rushtapakornchai and Vatanatangum,1985). It is considered that insensitive ChEs were not observed as the result.

As there were many kinds of ChE types in DBM, negatively correlated cross resistance like *Heliothis* (Brown and Bryson, 1992) and green rice leafhopper (Yamamoto *et al.*, 1993) was not observed. This shows potential of DBM to increase insecticide resistance, and the difference of the sensitivity depend on ChE genes from several to ten times more will first be selected by insecticides. Actually, the frequency of the ChE types in each field strain (Tukuba and Okinawa strains) was consistent with the levels of practical insecticide resistance. In recent years, insecticides to control DBM have been transferring to new insecticides which do not inhibit AChE. DBM has already developed resistance to those new insecticides. However, as insecticide resistance of DBM is unstable, it is possible that the sensitivity to insecticides not used for a long time has already recovered regionally. To prevent resistance to new insecticides and resurgence, it is needed to know regional level and intensity of insecticide resistance. The monitoring method described here can at least easily estimate insecticide resistance level of DBM to OPs and carbamates.

Acknowledgements

This work was supported in part by the International Research Fund by the Japan Society for the Promotion of Science, the Chemical Materials and Development Foundation and the Grant-in-Aid for Scientific Research (A) No. 07556014 by the Ministry of Education, Science and Culture, Japan.

References

Ankersmit,G.W. (1953). DDT-resistance in *Plutella maculipennis* (CURT.) (LEP.) in Java. *Bull. Ent. Res.* ***44:*** 421–426

Byrne, F. J and A. L.Devonshire (1993). Insensitive Acetylcholinesterase and Esterase Polymorphism in Susceptible and Resistant Populations of the Tobacco Whitefly *Bemisia tabaci* (Genn.). *Pestic. Biochem. Physiol.* ***45:***34–42

Brown, T. M and P. K. Bryson (1992). Selective inhibitors of methylparathion-resistant acetylcholinesterase from *Heliothis virescens. Pestic. Biochem. Physiol.* ***44:*** 155–164.

Chen, J. S.and C. N.Sun (1986). Resistance of diamondback moth (Lepidoptera:Plutellidae) to a combination of fenvalerate and piperonyl butoxide. *J. Econ. Entomol.* ***79:*** 22–30.

Ellman, G. L., K. D. Courtney, V. Andres and R. M. Featherstone (1961) A new and rapid colorimetric determination of acetylcholinesterase actibity. *Biochm. Pharmacol.****7:*** 88–95.

Fahmy, A. R., N. Sinchaisri and T. Miyata (1991). Development of chlorfluazuron resistance and pattern of cross resistance in the diamondback moth, *Plutella xylostella. J. Pesticide sci.* ***16:*** 665–672

Finney, D. J. (1971). Probit analysis. 3rd. ed. London: Cambrige Univ. Press, pp. 315.

Ffrench-constant, R. H.and B. C. Bonning (1989). Rapid microtitre plate test distinguishes insecticide resistant acetylcholinesterase genotypes in the mosquitoes *Anopheles albimanus, An.nigerrimus* and *Culex pipiens. Medic. Veteri. Entomol.* ***3:*** 9–16.

Miyata T.,T. Saito and V. Noppun(1986). Studies on the mechanism of diamondback moth resistance to insecticides. *In:* Diamondback Moth Management (N. S. Talekar and T. D. Griggs eds.), Shanhua, Taiwan: AVRDC, pp. 347–357.

Moores, G. D.and A. L. Devonshire (1984). Characterisation of insecticide- insensitive acetylcholinesterase : microcomputer based analysis of enzyme inhibition in homogenates of individual housefly (*Musca domestica*) heads. *Pestic. Biochem. Physiol.* ***21:*** 341–348.

Noppun, V., T. Miyata and T. Saito (1983). Susceptibility of four strain of the diamondback moth, *Plutella xylostella* L., against insecticides. *J. Pestic. Sci.* ***8:*** 595–599.

Noppun, V.,T. Miyata and T. Saito (1987). Cross resistance and synergism studies in the diamondback moth, *Plutella xylostella* L.(Lepidoptera: Yponomeutidae). *Appl. Entomol. Zool.* ***22(1):*** 98–104.

Perng, F. S., M. C. Yao, C. F. Hung and C. N. Sun (1988). Teflubenzuron resistance in diamondback moth (Lepidoptera:Plutellidae). *J. Econ. Entomol.* ***81:*** 1277–1282.

Rushtapakornchai, W. and A. Vattanatangum (1985). Present status of insecticidal control of diamondback moth in Thailand. *In:* Diamondback moth management. (N.S.Talekar and T.D.Griggs eds.), Tainan: AVRDC., pp. 307–312

Saito T., N. Sinchaisri, A. Vattanatungum, T. Miyata, W. Rushtapakornchai, O. Sarnthoy, P. Kienmeesuke, F. Nakasuji, Y. Tsubaki and B. Saympol. (1990). Challenge to diamondback moth resistance to insecticides. Proceedings of 3rd international conference on plant protection in the tropics, Genting Highlands, Pahang, *Malaysia, Vol. 3*, 157–164.

Sasaki, Y.(1982). Studies on insecticide resistance of the diamondback moth. In "Results on the Pest Control Studies (Annual report for 1982)". Jpn. Plant Prot. Assoc. Tokyo, pp. 92–102 (in Japanese).

Sinchaisri, N., T. Apajirakul and V. Noppun(1980). Evidence of resistance to insecticides of some key pests in Thailand. XVI Int. Cong. Ent. (Kyoto).

Sudderuddin, K. I. and P. F. Kok (1978). *FAO Plant Prot. Bull.* ***26:*** 53–57.

Sun, C. N., H. Chi and H. T. Feng (1978). Diamondback moth resistance to diazinon and methomyl in Taiwan. *J. Econ. Ent.* ***71:*** 551–554.

Tabashnik, B. E., N. L. Cushing, N. Finson and M. W. Johnson (1990). Field development of resistance to *Bacillus thuringensis* in diamondback moth (Lepidoptera: Plutellidae). *J. Econ. Entomol.* ***83:*** 1671–1676.

Tang, Z. H., K. Y. Gong and Z. P. You (1988). Present status and countermeasures of insecticide resistance in agricultural pests in China. *Pestic. Sci.* ***23:*** 189–198.

Yamamoto, I., N. Kyomura and Y. Takahashi (1993). Negatively correlated cross resistance : combinations of N-metylcarbamate with N-propylcarbamate or oxadiazolone for green rice leafhopper. *Archi. Insect. Biochem. Physiol.* ***22:*** 277–288.

Allozymic polymorphism among three populations of *Plutella xylostella*

Noran, A.M. & Tang, P.Y.

Department of Zoology, Faculty of Life Sciences, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia

Abstract

The interpopulation and intrapopulation genetic variations of three populations of the diamondback moth (DBM), *Plutella xylostella*, namely from Cameron Highlands (high altitude), Gombak (lowland) and MARDI (bred on artificial diet), were analysed through determination of allozymic polymorphism using horizontal starch gel electrophoresis. The aim was to detect variations arising from prolonged exposure to differences in diet, temperature, altitude and insecticides. Six out of eight loci tested, i.e., MDH-2, MDH-3, PGM, PGI, MPI and ME-2, were consistently observed. Four of these loci were polymorphic, with only MDH-2 and ME-2 being monomorphic, indicating that there were genetic variations within and between populations. The polymorphic index for the MARDI population was highest (0.112) followed by that of Gombak (0.091) and Cameron Highlands (0.090). However, not every population showed Hardy-Weinberg equilibrium, probably due to the population or sample size being limited. Only 6 genetic markers, namely, MDH-1, ME-1, MPI-AA (from Cameron Highlands), MPI-BC (from Gombak), PGM-AB and MPI-BD (from MARDI) were observed. The genetic identity between the Gombak and MARDI populations was highest (D=0.993), while the values between the Gombak and Cameron Highlands (D=0.896) and between those of MARDI and Cameron Highlands (D=0.872), respectively, were less. Hence, the MARDI and Gombak populations showed closer relationship compared to either with those of Cameron Highlands. It can be concluded that interpopulation variations may arise due to prolonged exposure to differences in temperature, altitude and insecticides use rather than variation in diet.

Key words: *Plutella xylostella*, allozymic polymorphism, starch gel electrophoresis

Introduction

Plutella xylostella, or the diamondback moth (DBM), is a cosmopolitan pest of Brassicae, including the wild and domestic species of Cruciferae and *Raphanus* (Hill, 1983). This study aims to survey the allozymic variations of three populations of DBM which may be affected by differences in their diet, environmental temperature and altitude and exposure to pesticides.

Materials and Methods

Sample source Thirty larvae were collected from each of three locations, namely a cabbage farm in Cameron Highlands, a lowland 'edible' rape leaf (*Brassica chinensis* var. *oleifera*) farm in Gombak and the MARDI "artificial diet" laboratory. The larvae were frozen at -80 °C with cryoprotectant (Richardson *et al*., 1986) until electrophoresis.

Electrophoretic analyses Each frozen larva was homogenized and the resulting extract was subjected to standard horizontal starch gel electrophoresis (Caprio, 1990). Nine enzymes, namely malate dehydrogenase (MDH), phosphoglucose mutase (PGM), phosphoglucose isomerase (PGI), mannose phosphate isomerase (MPI) and malic enzyme (ME), were surveyed. The protocols used in staining for PGM, PGI, MPI and ME were as described by Miles *et al*. (1980), while that for MDH was as described by Shaw and Prasad (1970).

Statistical analyses Allelic frequencies were calculated. Polymorphic locus was determined when the frequency of the commonest allele was less than or equal to 0.95. Chi square tests were used to examine the distribution of phenotypes in relation to their expected Hardy-Weinberg equilibrium distribution. Polymorphic index was calculated to compare the degree of polymorphism in each population (Marshall and Allard, 1970). Genetic distance (D) and genetic similarity (I) between two populations were calculated following Nei's method (1972) to estimate the degree of genetic divergence. A dendrogram was constructed to demonstrate the genetic relationships among the populations (Sneath and Sokal, 1973).

Results

Eight loci were determined based on the electrophoretic patterns of five enzymes. The electropherograms of the five enzymes are shown in *Figs. 1–6*. Based on the number of allozymic phenotypes for each population, the allelic frequencies were estimated for each locus as summarized in *Table 1*. Only four out of eight loci, namely, MDH-3, PGM, PGI and MPI were consistently found to be polymorphic while the other two loci (MDH-2 and ME-2) were monomorphic. The phenotypes of six genetic markers or loci, i.e., MDH-1, ME-1 and MPI-AA from Cameron Highlands, MPI-BC from Gombak,

Table 1 : Allelic frequencies of isozyme loci in *Plutella xylostella.*

Locus	Allele	Cameron Highlands	Gombak	MARDI
MDH-1	A			
MDH-2	A	0.5	0.5	0.5
	B	0.5	0.5	0.5
MDH-3	A	0.333	0.350	0.383
	B	0.667	0.650	0.617
PGM	A	0.333	0.033	0.100
	B	0.283	0.333	0.683
	C	0.383	0.633	0.517
PGI	A	0.150	0.117	0.133
	B	0.850	0.883	0.867
MPI	A	0.067	0.000	0.000
	B	0.100	0.100	0.133
	C	0.467	0.367	0.467
	D	0.367	0.533	0.400
ME-1				
ME-2	A	0.067	0.000	0.033
	B	0.933	1.000	0.967

PGM-AB and MPI-BD from MARDI, were detected. Based on the calculated results, only 1/3 of the surveyed loci from Cameron Highlands, 2/3s of the loci from Gombak and MARDI showed Hardy-Weinberg equilibrium, probably due to the limited population or sample size.

The polymorphic index values are shown in *Table 2.* The MARDI population showed the highest value followed, respectively, by the Gombak and Cameron Highlands populations. The results indicated that the highest genetic variation was among the MARDI population.

The genetic relationships between the three populations of DBM, based on the indices of genetic distance among them *(Table 3)*, indicated that the MARDI and Gombak populations had the closest relationship *(Fig 6)*. This means that interpopulation variations were more affected by differences in temperature, altitude and the effects of insecticides used rather than variations in diet

Discussion

Our preliminary survey of the larval body weights and lengths of the three populations of *Plutella xylostella*, i.e., from MARDI, Cameron Highlands and Gombak, showed significant differences ($p < 0.001$; n = 30), the ones from MARDI being heaviest (average 8.13 mg) and longest (9.26 mm) followed by the ones from Cameron Highlands and Gombak, respectively. This was not surprising since the MARDI population was cultivated on an artificial diet rich in nutrients and, furthermore, was not subjected to any stress like pesticides. Allozymic analyses were then carried out to determine the genetical basis for the differences observed.

When allozymic analyses were carried out, interestingly, the genetic identity between the Gombak and MARDI samples was highest compared to either with the Cameron Highlands samples, being furthest between those from C. Highlands and MARDI. This means that altitude and temperature differences have more influence on the alleles being expressed and that the allozymes studied are not linked to the genes coding for morphological variations like body weight and length. Analyses of more allozymes are, therefore, required for more conclusive evidence.

Among the allozymes analysed, the enzyme malate dehydrogenase (MDH) was found to be coded for by 3 loci, namely, MDH-1, MDH-2 and MDH-3, in contrast to only one locus consisting of 3 alleles, as reported earlier by Caprio (1990). Nevertheless, MDH was shown to be coded for by 3 loci in the fish *Clarias batrachus* (Ismail *et al*., 1989) and *Puntius* spp (Siraj *et al*., 1993), which is similar to our observation. Our observation that only the C. Highlands population, represented by three individuals, demonstrated the presence of MDH-1 locus indicated its rareness or low mutation rate and will require a larger population survey for it to be used as a genetic marker for this population.

Our finding that the enzyme phosphoglucomutase (PGM) was coded for by one polymorphic locus was identical to that reported by Caprio (1990) but we could only detect 3 alleles compared to 5 by Caprio. Sample size could be responsible for this discrepancy. The PGM-AB phenotype was only detected in the MARDI population and could be used as a genetic marker for that population.

Our finding on the polymorphic phosphoglucose isomerase (PGI) system was as reported by Caprio (1990), i.e., it was coded for by one locus and was a dimer. However, we only observed the PGI-BB and PGI-AB phenotypes in all three populations and not the PGI-AA, the heterozygosity being at a very low level.

We observed 7 phenotypes, namely MPI-AA, MPI-BB, MPI-CC, MPI-DD, MPI-BC, MPI-BD and MPI-CD, for mannose phosphate isomerase (MPI), a monomeric enzyme coded for by one locus. The

Table 3 : Matrix of genetic similarity and genetic distance

Population	1	2	3
1. Cameron Highlands	***	0.896	0.872
2. Gombak	0.110	****	0.993
3. MARDI	0.137	0.007	****

Table 2 : The polymorphic index of three populations of *Plutella xylostella*

Population	Polymorphic index						Mean
	MDH-2	MDH-3	PGM	PGI	MPI	ME-2	
Cameron Highlands	0.000	0.203	0.103	0.105	0.162	0.058	0.090
Gombak	0.000	0.205	0.122	0.089	0.132	0.000	0.091
MARDI	0.000	0.111	0.192	0.098	0.145	0.031	0.112

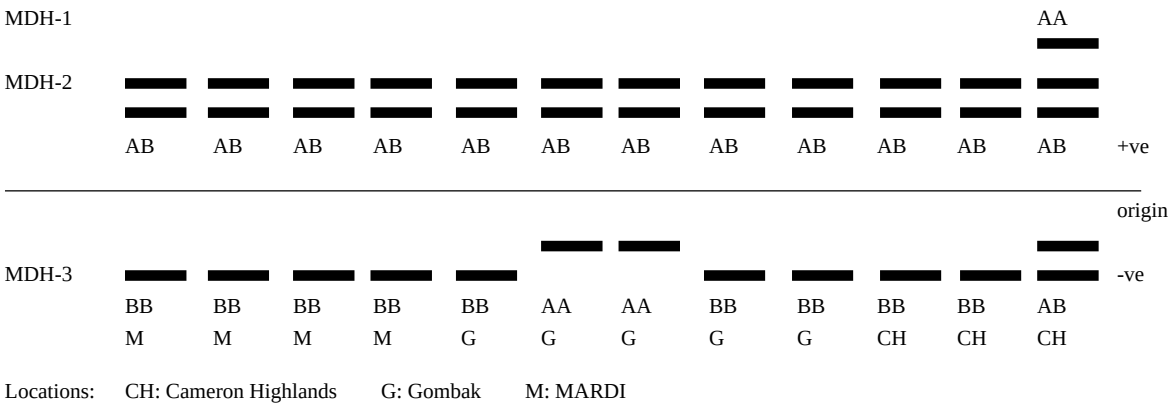


Fig. 1. Electropherogram of malate dehydrogenase (MDH) in Plutella xylostella

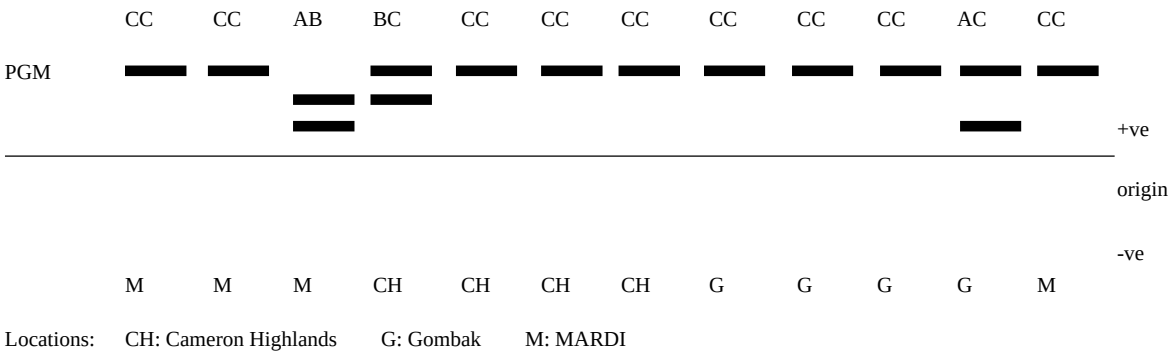


Figure 2. Electropherogram of phosphoglucose mutase (PGM) in Plutella xylostella

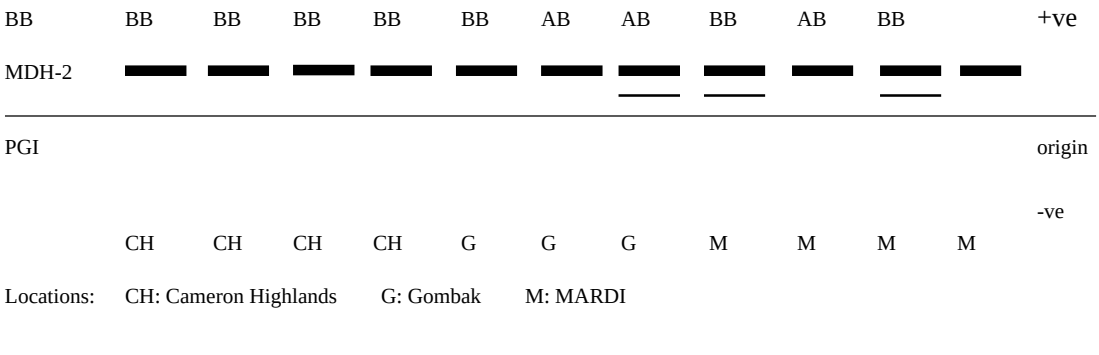


Figure 3. Electropherogram of phosphoglucose isomerase (PGI) in Plutella xylostella

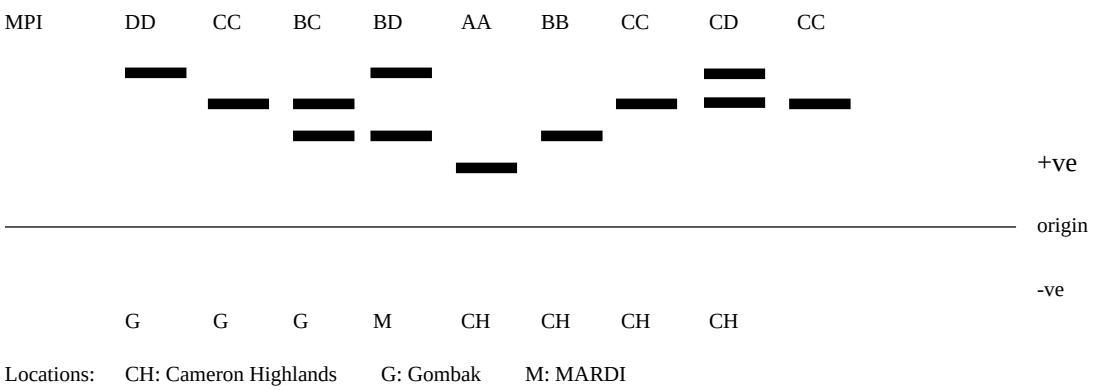


Figure 4. Electropherogram of mannose phosphate isomerase (MPI) in Plutella xylostella

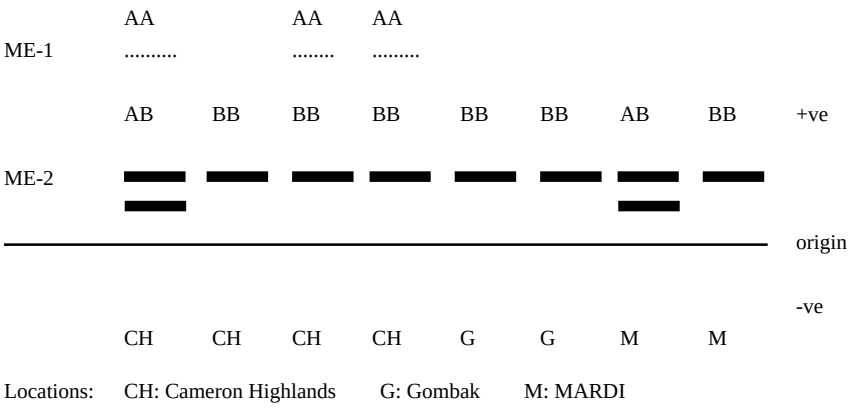


Figure 5. Electropherogram of malate enzyme (ME) in Plutella xylostella

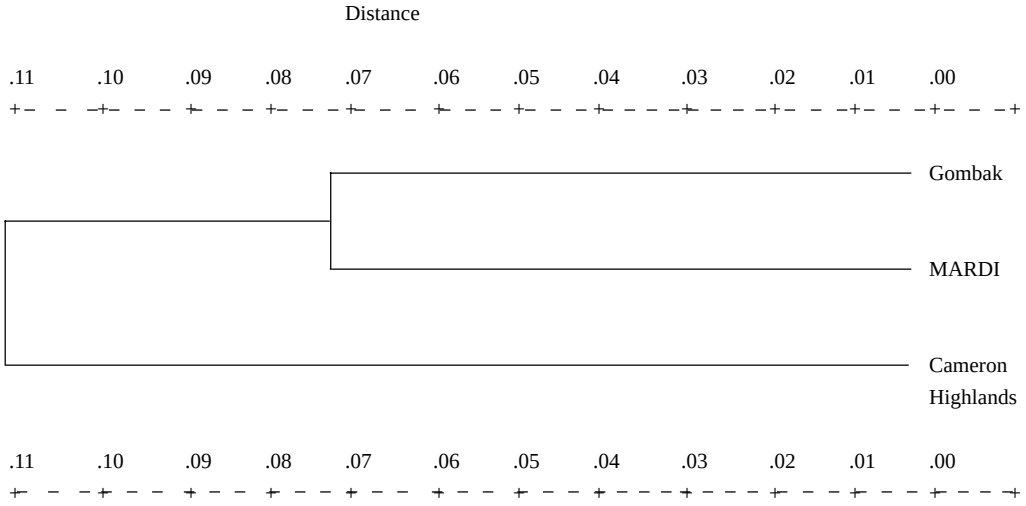


Figure 6. Dendrogram drawn based on the genetic distance between populations

heterozygosity was very low with MPI-BC only occurring in the Gombak and MPI-BD in the MARDI populations. The C allele was dominant at C. Highlands while the D allele at both Gombak and MARDI.

Finally, two loci, namely ME-1 and ME-2, for malate enzyme (ME), were detected for C. Highlands but only ME-2 was present in the other two populations. Only the phenotype ME-2BB was observed in all three populations, the locus being monomorphic.

Acknowledgements

We would like to thank Dr Hussan bin Abdul Kadir of MARDI for generously providing us with the artificial diet-bred *Plutella* samples and Universiti Kebangsaan Malaysia for the financial assistance and facilities.

References

Caprio, M. A. (1990). Gene flow as a factor in the evolution of insecticide resistance. *Ph.D Dissertation*, University of Hawaii at Manoa. 114 pp.

Hill, D. S. (1983). Agricultural insect pests of the tropics and their control. (Second Edition). Cambridge University Press, Cambridge. 746 pp.

Ismail, P., Daud, S.K. and Kajima,A. (1989). Genetic control of isozymes in the four catfish species. *Journal of Malaysian Applied Biology* ***18(1):*** 33–37

Marshall, D.R. and Allard, R.W. (1970). Isozyme polymorphisms in natural populations of *Avena fatua* and *A. barbata. Heredity* 25: 373–382

Miles, M. A., Lanham, S. M., De Souza, A. A. and Povoa, M. (1980). Further enzymic characters of *Trypanosoma cruzi* and their evaluation for strain identification. *Transaction of the Royal Society of Tropical Medicine and Hygiene* ***74(2):*** 221–237

Nei, M. (1972). Genetic distance between populations. *American Nature* ***106:*** 283–292

Richardson, B. J., Baverstock, P. R. and Adams, M. (1986). Allozyme electrophoresis. A handbook for animal systematics and population studies. Academic Press, Australlia. 410 pp.

Shaw, C. R. and Prasad, R. (1970). Starch gel electrophoresis of enzyme - a compilation of recipes. *Biochemical. Genetics* ***4:*** 297–320

Snead, P.H.A. and Sokal, R.R. (1973). Numerical taxonomy: The principles and practice of numerical classification. W.H. Freeman, San Franscisco. Pp 547

Exploratory survey of cabbage nurseries in Cameron Highlands, Malaysia

J.G.M. Vos and W.L. Reerink
International Institute of Biological Control, P.O. Box 210, 43409 UPM Serdang, Selangor, Malaysia

Abstract

An exploratory survey was done on cabbage farmers' nursery practices and crop health problems in the Cameron Highlands, Malaysia. Thirty cabbage farmers were interviewed. General farm management practices varied. Crop rotation was not always practised. Half of the farmers reported use of a permanent nursery. All farmers used certified seeds. Crop residues with disease symptoms were often found. Many farmers reported not to experience any crop health problems in nurseries, even though disease symptoms were observed. In the field, a major crop health problem is diamondback moth. Clubroot only sometimes causes problems. Black rot was found on most farms, but only few farmers reported it as a problem. Most farmers start applying pesticides in the nursery: often a cocktail spray consisting of at least one fungicide and one insecticide. Most farmers apply lime in the nursery as well as in the field. They are aware that liming can reduce clubroot. It was concluded that farmers did not associate black rot incidence in nurseries with disease problems in the field. Farmers did recognise clubroot symptoms in nurseries, but are not aware that it is a soil-borne disease. The survey conclusions need to be verified in a formal survey covering more farmers.

Key words: Cabbage, nursery, survey, crop health, Malaysia

Introduction

Plant raising techniques are assumed to affect crop health and crop performance after transplanting. In the Cameron Highlands, Malaysia, problems with cabbage crop health in the field, especially with soil-borne diseases such as black rot and clubroot, may originate in the nursery. If so, crop health management programmes should start at or even before preparing nurseries. An exploratory survey was done to obtain information about cabbage farmers' nursery practices and incidence of cabbage crop health problems in nurseries.

Methodology

Between January and May 1996, 30 cabbage farmers in the Northern, Central and Southern zones of the Cameron Highlands were interviewed regarding general farm management practices, cabbage nursery practices and cabbage crop health problems, both in the nursery and in the field. Whenever possible, cabbage nurseries and fields were visited and incidence of diseases, particularly black rot and clubroot, observed.

Results

The average farm size was 0.8 ha. General farm management practices varied considerably. Farmers reported to plant cabbage from once in 2 years up to 4 times a year in the same field, which means that crop rotation is not always practised. In some farms, cabbage was intercropped with onion. Half of the farmers reported use of a permanent nursery to raise cabbage seedlings. All farmers used certified seeds. Crop residues with disease symptoms were often found in and around the fields (in 18 of the 30 farms visited), indicating lack of sanitation.

Out of 30 farmers, 13 reported not to experience any crop health problems in cabbage nurseries *(Table 1)*. However, half of the observed nurseries showed black rot infection. In the field, farmers indicated that a major crop health problem is diamondback moth. Clubroot was reported to only sometimes cause problems. Of the other diseases, black rot was observed on most farms, but only 4 out of 30 farmers reported black rot as a problem.

For the management of pests (including diseases – *Table 2*), all farmers reported to apply pesticides in the field: often a cocktail spray consisting of at least a fungicide and an insecticide. Twenty-five farmers already start to use pesticides in the nursery. Spray intervals range from 4 to 7 days. The pesticides are applied to prevent and/or control diamondback moth, armyworm, cutworm, damping-off, downy mildew, black rot and clubroot. Most farmers apply lime in the nursery. In the field, 23 out of 30 farmers apply lime regularly, at least once a year. They are aware that liming can reduce clubroot. Some farmers in the Northern and Southern zone indicated that certain cabbage cultivars are less susceptible to black rot than others.

When further questioned about disease management, some contradictions were noticed. Most farmers said that black rot infected seedlings can grow into healthy plants in the field. Apparently, they are unaware of the spread of black rot through infected plant material, soil and irrigation water. Some farmers argued that black rot is caused by rain. On the other

Table 1. Number of farmers, out of 30, indicating problems with cabbage crop health in the nursery and in the field during an exploratory survey in Cameron Highlands, Malaysia, between January and May 1996

Crop health problem	Before transplanting	After transplanting
Insect pests:		
Diamondback moth	6	27
Cutworm	0	2
Others	0	9
Diseases:		
Black rot	4	4
Clubroot	1	1
Damping-off	5	0
Downy mildew	4	0
Soft rot	0	3
No problems	13	0

Table 2: Number of farmers, out of 30, indicating to conduct cabbage disease management practices in the nursery and in the field during an exploratory survey in Cameron Highlands, Malaysia, between January and May 1996

Disease management	# Farmers practising
Use certified seeds	30
Select healthy seedlings	5
Practise nursery rotation	15
Apply lime	
in nursery	22
in field	23
Practise crop rotation	16
Practise sanitation	12
Apply pesticides	
in nursery	25
in field	30

hand, farmers did argue that seedlings with clubroot symptoms should not be planted in the field.

Preliminary conclusions

Based on the sample size of only 30 farmers, general conclusions regarding cabbage nurseries in Cameron Highlands cannot be drawn. The following, preliminary conclusions are proposed and need to be verified in a formal survey:

- Farmers do not associate black rot incidence in nurseries with disease problems in the field. Nevertheless, pesticides (including fungicides) are used during plant raising by most farmers.
- Farmers discard seedlings with clubroot symptoms during transplanting, but are unaware that it is a soil-borne disease. They do not know that the disease can spread by soil, even when only symptomless seedlings are planted.
- In order to be able to manage cabbage diseases, farmers need to appreciate processes such as disease spread by infected plant material, soil and irrigation water and thereby better understand the benefits of, among others, sanitation measures and crop rotation.

Entomopathogenic nematodes against foliage feeding crucifer pests in the tropics

Judy M. Mason and Denis J. Wright

Imperial College of Science, Technology & Medicine, Department of Biology, Silwood Park, Ascot, Berkshire, SL5 7PY, UK

Abstract

Use of entomopathogenic nematodes against foliage pests is commonly perceived to be limited by their tolerance to temperature, desiccation and UV radiation. The effect of these abiotic factors on the infective juveniles (ijs) of two isolates of *Steinernema* spp. (SSL85), two isolates of *Steinernema* spp. (M87), *Heterorhabditis* n.sp. and *H. indicus* recovered from selected sites within Peninsular Malaysia was examined. Infectivity at different temperatures was found to differ both within and between species, with optimal infection generally at 25 °C. Desiccation studies revealed more marked differences between the isolates. For example, at 80% relative humidity, survival of approximately 51% of ijs of *Steinernema* spp. (SSL85/25) was observed, compared with 13% for *Steinernema* spp. (M87/45). Prior exposure of ijs suspended in water droplets to simulated solar radiation resulted in a general decline in % mortality of *Plutella xylostella* larvae in subsequent bioassays but no marked reduction in mean infection. These preliminary studies are encouraging as they suggest that entomopathogenic nematodes can tolerate, within defined limits, the major abiotic factors faced in the foliar environment. The results are discussed in terms of application under foliar conditions.

Key words: Entomopathogenic nematodes; temperature; desiccation; solar radiation; *Plutella xylostella*.

Introduction

With both the increase of pesticide resistance and greater awareness of the environmental impact of pesticides on the environment, alternative control agents for employment in the IPM of the major lepidopteran pests of crucifers are continually being sought. One such alternative is entomopathogenic nematodes.

Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) have been used with varying degrees of success in the biocontrol of a wide range of insect pests. Success has been greatest against soil-dwelling insects (e.g. Klein, 1990) and insects of cryptic habitats (e.g. Begley, 1990). Use of entomopathogenic nematodes against foliar insects has been limited, and has by and large been unsuccessful, with few exceptions (e.g. Begley, 1990). The reasons generally quoted for lack of success in this more hostile environment are desiccation, UV radiation and temperature stress caused to the nematodes.

In any biocontrol programme, one of the most important things is the selection of the most suitable isolate/species of nematode. This selection process must encompass the entire process ranging from ease of mass culture through to efficacy under field conditions. The entomopathogenic nematodes used in current study were isolated in Malaysia (Mason *et al*., 1996) and selected following mass screening of the infective juveniles (ijs) against third instar larvae of *Plutella xylostella*, one of the major lepidopteran pests of crucifers in Malaysia. The results of a series of preliminary experiments conducted to assess the effect of the major abiotic factors on the ijs of these isolates are presented below.

Materials and Methods

Nematode cultures

Six isolates were assessed: two of *Steinernema* spp. (SSL85: 25 & 43); two of *Steinernema* spp. (M87: 3 & 45); *Heterorhabditis indicus* and *Heterorhabditis* sp. All cultures were maintained at 20 °C on late instar larvae of *Galleria mellonella* L. (The Mealworm Co., Sheffield, U.K.), with ijs being used within six days of emergence.

Culturing of P. xylostella

A UK isolate of *P. xylostella* (Rothamstead insecticide susceptible strain) was obtained from Dr. M. Furlong (IACR-Rothamstead, Harpenden, Herts., UK). Cultures were maintained at 20 °C on 4–6 week-old Chinese cabbage, *Brassica oleracea* var. *capitata* ('Tip Top').

Effect of temperature on infection

The sand tube assay of Fan & Hominick (1991) was used to assess infectivity of ijs at a range of temperatures: 10, 15, 20, 25, 30, 35 and 40 °C. To 25ml of moist sand in 30ml universal tubes, 200 ijs suspended in 1ml of tap water were added. One late instar *G. mellonella* larva was added per tube. A screw top lid was fitted to each tube and the tubes inverted to ensure contact between the larva and sand. For each

nematode isolate, there were 20 tubes per test temperature. Tubes were incubated at the test temperature for 72 hours. The larvae were then removed, washed in tap water and further incubated at 20 °C (as required) prior to dissection. Both prevalence (percent insect mortality) and intensity of infection were assessed.

Desiccation tolerance

Desiccation tolerance was examined at 25 °C at two relative humidities (r.h.): 40% and 80%. Infective juveniles were desiccated according to the method of Perry (1977). Approximately 50–100 ijs suspended in a small volume of distilled water were pippetted onto a glass microscope slide. Excess water was removed using filter paper. The slide was then immediately placed in the r.h. chamber. Relative humidities were maintained using glycerol/water solutions (Grover & Nicol, 1940). There were five replicates per treatment. After the required period of desiccation, slides were removed and flooded with distilled water. The re-suspended ijs were assessed after 24 hours and the number of living nematodes recorded. Those ijs not responding to mechanical stimulation were considered to be dead.

Exposure to simulated tropical sunlight

The effect of exposure to simulated tropical sunlight was assessed by irradiating the ijs using a 1000W solar simulator (Oriel Corp., Stratford, CT, USA). The test arena consisted of a 5cm diameter Petri dish in which a Chinese cabbage leaf disk (5cm diameter) was embedded on top of 1% agar (1–2mm depth). Infective juveniles were applied to the leaf disks in 1µl droplets. Thirty droplets were pipetted evenly onto each leaf disk, giving approximately 200 ijs per leaf disk. Exposure to simulated tropical sunlight was for 0, 5, 10, 15, 20 and 25 minutes with five replicates per exposure time. Following exposure, four, third instar *P. xylostella* larvae were added to each arena to assess infectivity of the irradiated ijs. The dishes were sealed and incubated at 25 °C for 48 hours. Insect mortality (prevalence) and intensity of infection were then assessed. Controls were as above with the exception that no nematodes were added to the droplets.

Statistical analysis

All analyses were performed using the statistical package GLIM (Royal Statistical Society, London, 1985), allowing generalised linear modelling to be conducted. Analyses of variance and analyses of covariance were conducted using the binomial error distribution with logit link function, allowing significance to be tested using c^2 values (Crawley, 1993). Overdispersion was corrected for as required by either adjusting the scale parameter (equal sample sizes) or using Williams' procedure (unequal sample sizes), allowing F-tests to be used to test for significance (Crawley, 1993). Significance is reported at the 5% level.

Results

Effect of temperature on infection

Infection occurred within the temperature range of 15–35 °C, with no infection at either 10 °C or 40 °C. Within this range, percent mortality of *G. mellonella* larvae displayed little variation with temperature (results not shown). In contrast, the mean number of ijs infecting *G. mellonella* larvae varied significantly across the temperature range *(Figure 1)*, with most isolates displaying optimal infection at 25 °C. These results are encouraging as they show that ijs of each of the isolates assessed can infect (and cause mortality) at temperatures likely to be experienced in both highland and lowland crucifer production areas in Malaysia.

Desiccation tolerance

Survival of ijs following desiccation at 40% r.h. was not significantly different between the nematode isolates ($p>0.05$) *(Figure 2)*. All isolates could survive desiccation at 40% r.h. for at least 30 minutes, with the exception of *Steinernema* sp. (M87/45). Although survival was not significantly ($p>0.05$) different between the isolates, some interesting trends emerge. For example, both heterorhabditid species showed markedly high survival at 10 minutes (over 90%) followed by a massive decline to approximately 30% survival at 20 minutes, with survival then tailing off. In contrast, the steinernematids generally showed a large decline in survival after only five minutes, with further decline in survival being more gradual. *Steinernema* sp. (M87/3) displayed a gradual decline in survival: nearly 70% of the ijs surviving at 20 minutes, although by 30 minutes less than 3% were alive.

In marked contrast to the large drops in survival noted at 40% r.h., the decline in desiccation survival at 80% r.h. was more gradual *(Figure 3)*. For example, survival after 30 minutes was still over 50% for each of the isolates. Desiccation survival was not significantly ($p>0.05$) different between the isolates.

Exposure to simulated tropical sunlight

Exposures of 15 minutes and longer resulted in the evaporation of all visible water from the droplets containing the ijs. The results for percent mortality and mean infection (*Figures 4* and *5*, respectively) show that the ijs were capable of infecting and causing appreciable mortality of third instar *P. xylostella* following exposure to simulated sunlight for at least 10 minutes after all visible water had evaporated. Even allowing for the variability displayed in the data for percent mortality, the overall trend with increasing time was for a reduction in percent mortality *(Figure 4)*. Mean infection *(Figure 5)*, in contrast to % mortality, remained relatively constant over time [except *Steinernema* sp. (M87/3)].

Discussion

The results from the present laboratory study suggest that ijs of selected isolates/species of entomo-

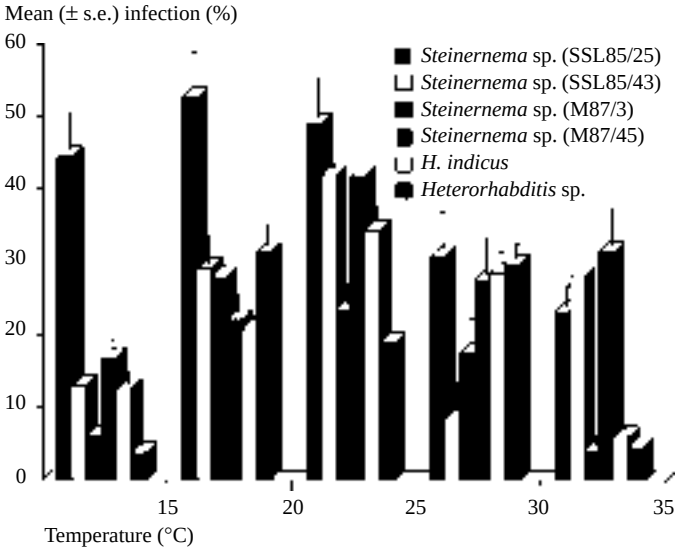


Figure 1. The effect of temperature on mean infection (%) of Galleria mellonella by Malaysian entomopathogenic nematodes (n=20)

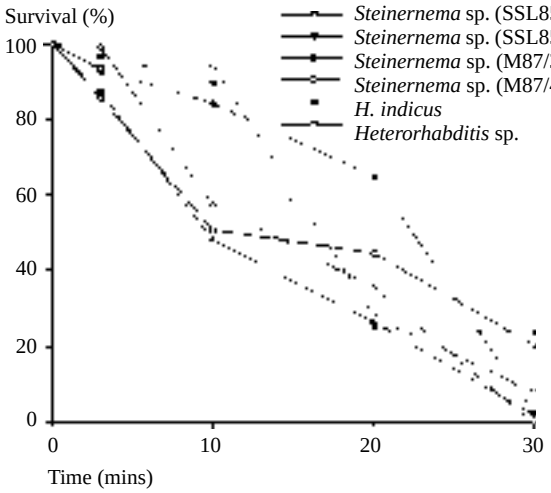


Figure 2. Survival (%) of Malaysian entomopathogenic nematodes following desiccation at 40% RH on glass slides

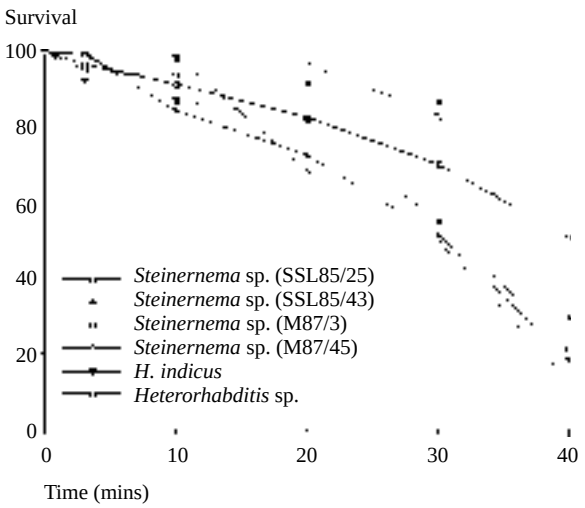


Figure 3. Survival (%) of Malaysian entomopathogenic nematodes following desiccation at 80% RH on glass slides

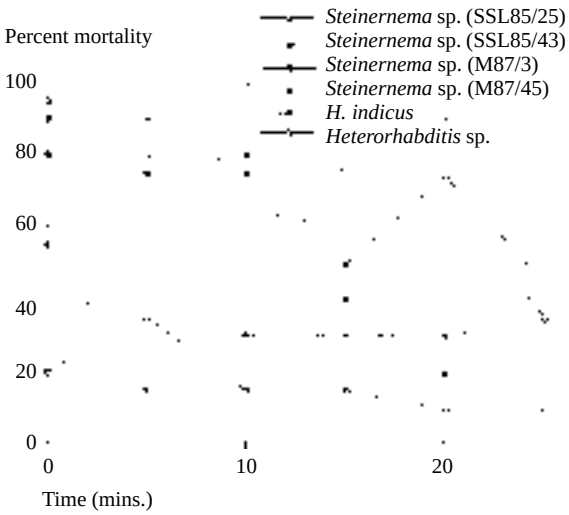


Figure 4. Mortality (%) of third instar Plutella xylostella larvae by entomopathogenic nematodes (in 1µl droplets) exposed to simulated tropical sunlight for different times (n=20)

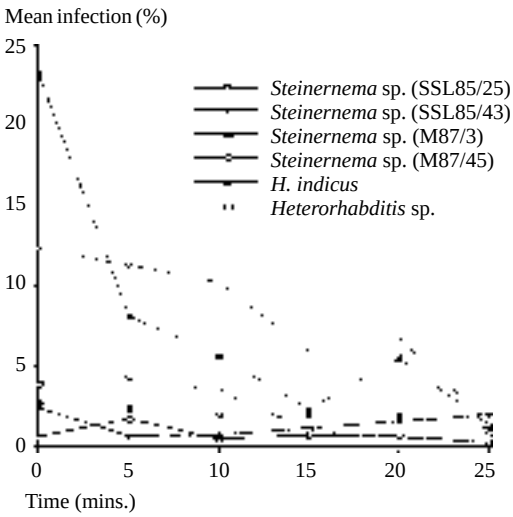


Figure 5. Mean infection (%) of third instar Plutella xylostella by entomopathogenic nematodes (in 1 µl droplets) exposed to simulated tropical sunlight for different times (n=20)

pathogenic nematodes have the potential for application in the foliar environment. For example, the temperature range for infection of *P. xylostella* larvae encompasses the range likely to be encountered in Malaysia – in either highland or lowland crucifer growing regions. Although surface temperatures on foliage, are likely to be higher (40 °C or more) due to exposure to direct sunlight, this, in terms of field efficacy, can be overcome by carefully timing the application to be early in the morning or in the late afternoon/early evening.

Similarly, although the method used to assess desiccation survival does not faithfully mirror field conditions, the results obtained, especially at 80% r.h., are very encouraging as they show that survival of the ijs is approximately 13–51% after 40 minutes. The glass slide method used induces a very rapid dehydration of the ijs and while rapid dehydration will be norm in the foliar environment it may not be as extreme due to the influence of the micro-climate surrounding the leaves. Indeed, both Glazer (1992) and Bauer *et al.* (1995) have observed elevated survival (in terms of infectivity) of entomopathogenic nematodes on leaf surfaces with dense pubescence. The present results, using this rapid dehydration and rehydration method, further suggest that, following evaporation of droplets under field conditions, it may be feasible to re-spray using water to rehydrate the desiccated ijs and perhaps increase the efficacy of the ijs. Such a strategy will only be feasible if low volume application methods are used (Lello *et al*., 1996). Further studies will be conducted into desiccation survival of the ijs using methods which reflect foliar conditions following field application.

The present results also suggest that ijs can survive, infect and cause lethal infections following exposure to simulated tropical sunlight, at least under defined conditions. These results differ from earlier publications (e.g. Gaugler *et al.*, 1992) but this can be accounted for by the different methods used. The present study applied ijs in discrete water droplets, mirroring to a certain extent the situation following field application. It was encouraging that the ijs caused infection and mortality even after the water droplets had evaporated. This may be due to maintenance of a high r.h. following irradiation, so that although there was no visible water, the conditions at the leaf surface-air surface interface (i.e. 'micro-climate') remained favourable for infection.

Acknowledgement

This research is supported by the Leverhulme Trust.

References

Bauer, M. E., Kaya, H. K. & Thurston, G. S. (1995). Factors affecting entomopathogenic nematode infection of *Plutella xylostella* on a leaf surface. *Entomologia Experimentalis et Applicata* ***77:*** 239–250.

Begley, J. W. (1990). Efficacy against insects in habitats other than soil. In *Entomopathogenic Nematodes in Biological Control* (ed. R.Gaugler & H.K.Kaya) pp 215–231 CRC Press.

Crawley, M. J. (1993). GLIM for Ecologists. Blackwell Scientific Publications, Oxford, UK, 379pp.

Fan X. and Hominick, W. M. (1991). Efficiency of the *Galleria* (Wax Moth) baiting technique for recovering infective stages of entomopathogenic rhabditids (Steinernematidae or Heterorhabditidae) from sand and soil. *Revue de Nematolgie* ***14:*** 381–387.

Gaugler, R., Bednarek, A. and Campbell, J. F. (1992). Ultraviolet inactivation of heterorhabditid and steinernematid nematodes. *Journal of Invertebrate Pathology* ***59:*** 155–160.

Glazer, L. (1992). Survival and efficacy of *Steinernema carpocapsae* in an exposed environment. *Biocontrol Science and Technology* ***2:*** 101–107.

Grover, D. W. and Nicol, J. M. (1940). The vapour pressure of glycerine solutions at 20°. *Journal of the Society of Chemical Industry* ***59:*** 175–177.

Klein, MG. (1990). Efficacy against soil-inhabiting pests. In *Entomopathogenic Nematodes in Biological Control* (ed. R.Gaugler and H.K.Kaya) pp 195–214 CRC Press.

Lello, E. R., Patel, M. N., Matthews, G. A. and Wright, D. J. (1996). Application technology for entomopathogenic nematodes against foliar pests. *Crop Protection* ***15:*** 567–574.

Mason, J. M. Razak, A. R. and Wright, D. J. (1996). The recovery of entomopathogenic nematodes from selected areas within Peninsular Malaysia. *Journal of Helminthology* ***70:*** (in press).

Perry, R. (1977). Desiccation survival of larval and adult stages of the plant parasitic nematodes *Ditylenchus dipsaci* and *D.myceliophagus* during desiccation and rehydration. *Parasitology* ***74:*** 139–148

Baseline data for field monitoring system for *Bacillus thuringiensis* resistance in Tamil Nadu (India) *Plutella xylostella* (L.) population

J. Chandrasekaran and A. Regupathy
Tamil Nadu Agricultural University, Coimbatore – 641 003, India

Abstract

The leafdip (IRAC method No. 7) has been used to determine the variability in baseline susceptible response to *Bacillus thuringiensis* in diamondback moth (DBM) field populations. Cabbage leaf discs of 6 cm dia. were dipped for 30 seconds in the commercial *B. thuringiensis* Berliner subspecies *kurstaki* (BTKR) formulation Biobit 50 WP (32000 IU/mg) dilutions in distilled water containing 0.1% of sticker/ spreader (Sandovit) and left to air dry. The field populations from the distinct cabbage growing regions of Tamil Nadu and laboratory reared F_{25} populations were tested. Slopes were 2.24, 1.74, 2.53 and 1.82 and LC_{50}s were 1.60, 1.32, 1.51 and 2.91 ppm a.i. for lab and field populations from Palada, Thombilipalayam and Oddanchatram, respectively. The early indications are that 18 ppm a.i. (99% kill) may be an appropriate discrimination dose for third instar of Tamil Nadu DBM, although more populations need to be screened.

Key words: Field monitoring system, Bt resistance, *Plutella xylostella*

Introduction

Incidences of damage caused by the diamondback moth (DBM) *Plutella xylostella* have gradually increased in Tamil Nadu in accordance with the cultivation of cabbage and cauliflower crops all the year round in different agroclimatic regions. In the 1970's, this pest could be easily controlled with organophosphates (OP) such as quinalphos, monocrotophos etc. However in the mid 1980's, the effectiveness of several OP's for the control of this pest started declining in various places like Ooty, Coimbatore and Madurai *(Figure 1)*. The initial field control failures with OP was overcome with the use of synthetic pyrethroids introduced in 1982. However, incidence of high resistance took place a few years after their introduction. A systematic resistance monitoring programme was initiated and is currently being continued reveals high level of resistance to fenvelerate and quinalphos. The resistance to newly introduced cartap hydrochloride is also on the line and cross resistance to carbosulfan which has not yet been put in the market is discernable. The biorational agents, especially, the products based on *Bacillus thuringiensis* Berliner (Bt) are suggested for the resistance management. Recently, farmers started to use more and more of *B. thuringiensis* formulations due to DBM resistance to conventional insecticides. Resistance was thought to be unlikely for *B. thuringiensis*, but through laboratory selection increased resistance was shown (Georghiou, 1989). The present study was conducted to create log-dose probit mortality (LDPM) for *B. thuringiensis*, using one laboratory reared and three field populations of DBM.

Material and Methods

The *B. thuringiensis* formulation (Biobit 32 000 IU/ mg) obtained from M/s Rallies India Ltd, Bangalore was used in the study. The laboratory reared F_{25} population of DBM (without exposure to any insecticide) originally collected from the fields of Horticultural Research Station, Tamil Nadu Agricultural University, Vijayanagaram from Ooty during the early part of 1990 and was used. The field populations tested were from Palada, a place around 10 km away from Ooty, Ambilikkai near Oddanchatram in Madurai district, where *B. thuringiensis* is being used regularly since 1990, and Thombilipalayam, a place near Coimbatore, where the usage of *B. thuringiensis* began from 1993–94.

Three-day old third instar larvae (ca. 0.5 ± 0.10 cm, 1.80 ± 0.30 mg) were used in the bioassay. The F_1 of the field collected larvae were used. The method of assay used was IRAC No. 7 whereby discs of 6 cm diameter were cut from fully expanded leaves of cabbage variety Quisto grown from seed in the greenhouse. Discs were dipped for 5 sec in distilled water dilutions of *B. thuringiensis* formulation. After treatment the discs are hung vertically to dry at laboratory temperature (29 ± 2 °C) for 2h and placed in plastic cups over a moistened filter paper. Ten unsexed DBM larvae were released per leaf disc and allowed to feed. Mortality was recorded at 48h and larvae failed to show any co-ordinated movement on prodding with a camel hair brush were considered dead. In all five concentrations were used. The treatments were replicated by repeating the experiment upto a week so as to have at least 100 to 150 larvae exposed to each concentration. The LDPM was worked out using probit analysis (Finney, 1971).

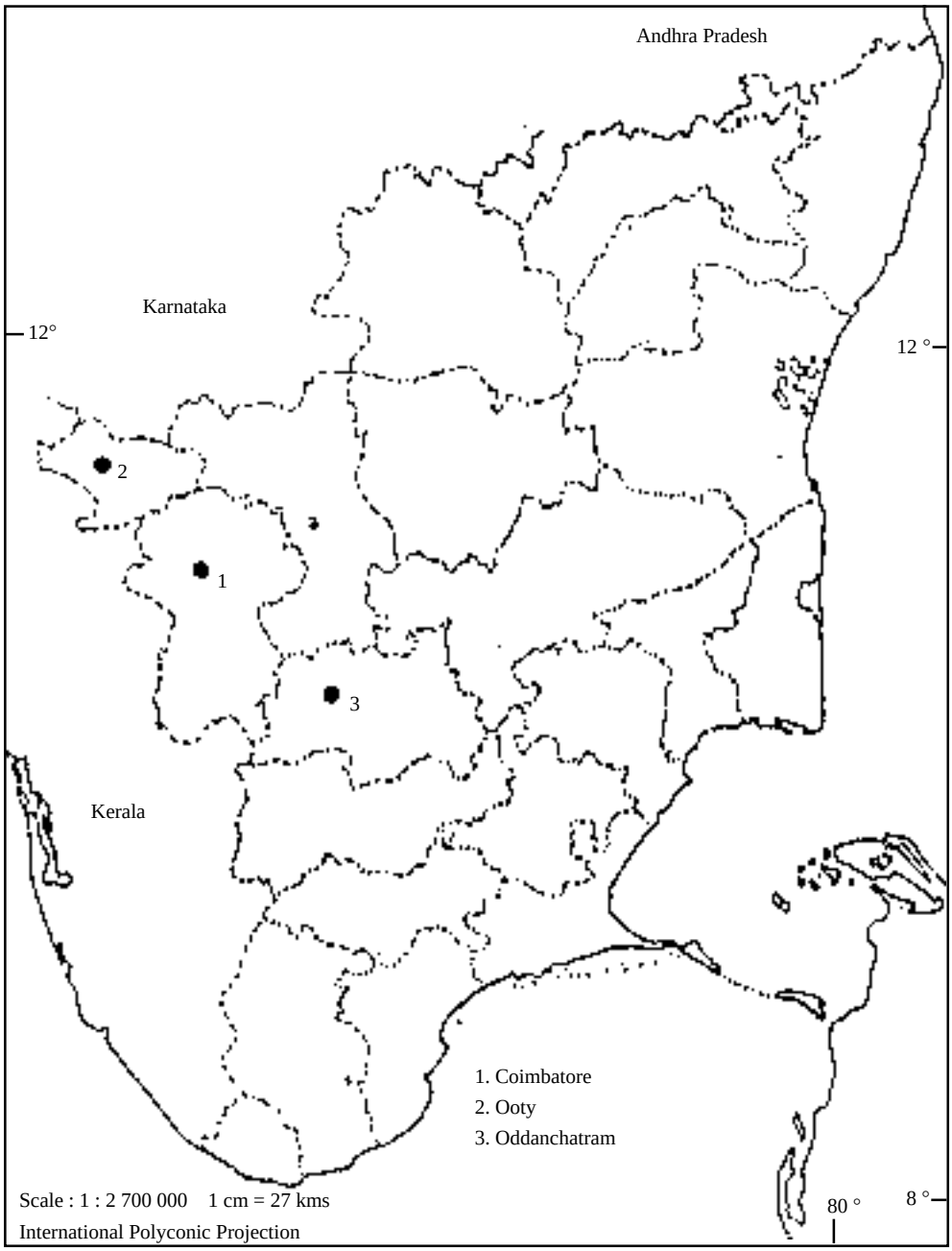


Figure 1. Sampling locations for Plutella xylostella collection in Tamil Nadu

Table 1. Susceptibility of *P. xylostella* to B.t. in Tamil Nadu (India)

Population	X	β	$\times^2$	LC_{50}	Fiducial limit		LC_{99}
				(ppm)	lower	upper	(ppm)
F_{25} Vijayanagaram	2.280	2.24	1.05	1.638	1.632	1.644	17.97
Palada	3.047	1.740	1.251	1.326	1.155	1.497	28.94
Oddanchatram	2.335	1.820	0.150	2.913	2.803	3.023	55.53
Thombilipalayam	2.020	2.530	0.330	1.506	1.504	1.508	12.55

Results and Discussion

The parameters of LDPM are presented in the *Table 1*. The slope value varied from 1.740 to 2.530. The steepest slope of 2.530 was recorded for Thombilipalayam population followed by 2.240 for F_{25} Vijayanagaram. The LC_{50} values varied from 1.326 mg/lit in Palada to 2.913 mg/lit. Correspondingly, the LC_{99} values were 17.97, 28.94, 55.53 and 12.55 mg/lit, respectively, for F_{25} Vijayanagaram, Palada, Oddanchatram and Thombilipalayam populations. The susceptible LAB-P populations recorded LC_{50} of 5.1 mg (AI)/lit while the resistant NO-Q recorded 3600 mg (AI)/lit (Tabashnik *et al*, 1992). The LC_{50} values ranged from 0.32 to 3.9 ppm for different populations, tested between 1985–1988 in Wakayama prefecture located in the Southwest Japan (Morista *et al*, 1992)

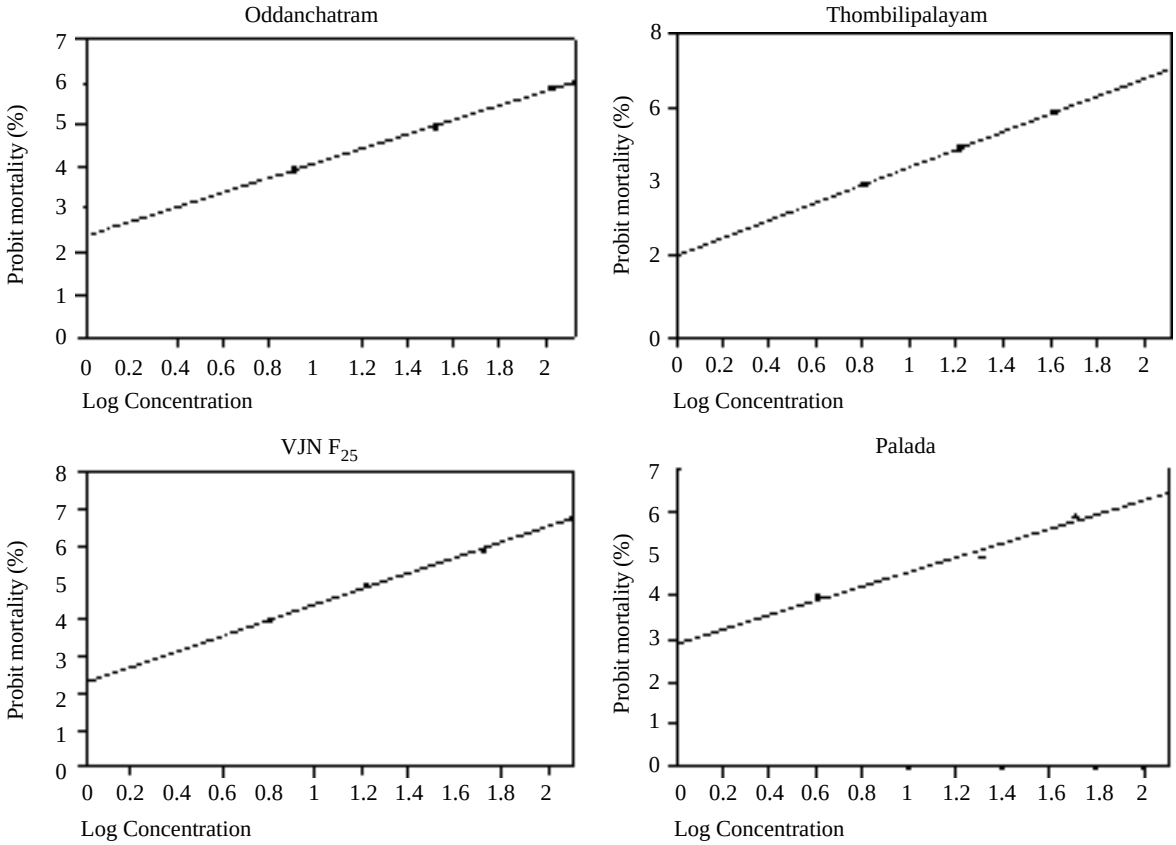


Figure 2. LDPM lines for four DBM populations

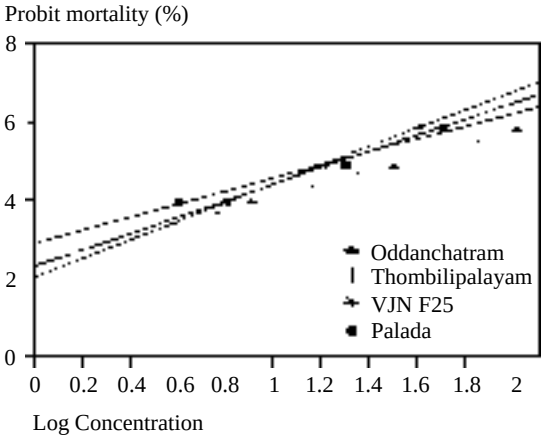


Figure 3. LDPM lines of four DBM populations

while the same was increasing from 0.3 ppm to 41.7 ppm from October 1985 to November 1990 (Adachi and Futai, 1992). The present estimate is less than that of the LAB-P susceptible DBM used by Tabashnik *et al* (1992) but more compared to Wakayama prefecture populations. Considering the Bt usage, the Oddanchatram population recorded the highest value of 2.913 mg (AI) lit., while the Palada population recorded the minimum value of 1.326 mg (AI)/lit. However, as the slope was more steep in Thombilipalayam and the F_{25} laboratory populations, the LC_{99} was minimum, 12.55 and 17.97 mg (AI)/lit respectively and the dose 12.55 mg (AI)/lit will be ideal for monitoring work.

Acknowledgement

The authors are thankful to M/S. Rallis India Ltd for financial support.

References

Adachi, T. and Futai, K. (1992). Changes in Insecticide Susceptibility of the Diamondback moth in Hyogo, Japan. *JARQ*, ***26:*** 144–151.

Finney, D. J. (1971). *Probit analysis*, 3rd ed. Cambridge University Press. Cambridge. pp. 390.

Georghiou, G. P. (1989). Implications of potential resistance to bio-pesticides In: *Proceedings of a conference : bio-technology, biological pesticides and novel plant pest resistance for insect pest management*, (D.W. Roberts & R.R. Granados. eds.) Boyce Thompson Institute for Plant Research, 1988. Ithaca. New York.

Morishita, M., Azuma, K. and Yano, S. (1992). Changes in insecticide susceptibility of the diamondback moth in Wakayama, Japan. *JARQ*, ***26:*** 139–143.

Tabashnik, B. E., Schwartz, J.M., Finson. N. and Johnson, M. W. (1992). Inheritance of resistance to *Bacillus thuringiensis* in Diamondback moth (Lepidoptera : Plutellidae). *Journal of Economic Entomology*. ***85(4):*** 1046–1055.

Sampling adult male populations of *Hellula undalis* (Lepidoptera:Pyralidae) in cabbage using virgin-females baited sticky trap

A. Sivapragasam, Asma Ahmad and Rafi Abdullah

Strategic, Environment and Natural Resources Research Center, MARDI, Serdang, Selangor, Malaysia

Abstract

The activity of adult males of *Hellula undalis* (Fabr.) in a cabbage field was determined using a sticky trap baited with two laboratory-reared one-day old virgin females. The daily activity pattern of *H. undalis*, based on the number of males trapped, was also determined at hourly intervals in four crop areas. The relationship between trap catches, larval populations on the cabbage plants and amount of rainfall was examined.

Trap catches of adult males of *H. undalis* were consistently high suggesting that immigration could be an important factor influencing the dynamics of the *H. undalis* population on cabbage. There was a poor relationship (r = 0.03) between trap catches and larval populations in the field. Rain also did not influence trap catches. Daily activity of male moths, based on trap catches, suggested a bimodal pattern with a higher peak between 5.30 h to 6.30 h and a relatively smaller around 20.30 h.

Key words: *Hellula undalis*, cabbage, virgin-female, trap, activity

Introduction

Amongst the insect pests which inflict serious damage to head cabbage, *Brassica oleracea* var. *capitata*, particularly in the lowlands of Malaysia, is the cabbage webworm (CWW), *Hellula undalis* (Fabr.) (Syed *et al*, 1992; Sivapragasam, 1994). Crop life tables on cabbage indicated that this pest is responsible for about 41 percent of total mortality of plants during the preheading stage (Sivapragasam, 1994). One of the major problems contributing to the pest status of *H. undalis* is the fact that there is no economic threshold level for *H. undalis* to initiate insecticidal treatments. Thus, a single larva boring into the growing shoot of the cabbage plant during the preheading stage, can result in either the death of the plant or produce two or more small-sized heads that are not marketable. Consequently, control measures initiated against this pest are preemptive and employed chemical insecticides on a weekly or twice weekly basis. The frequent use of pesticides elevates the cost of production and increases selection pressure towards resistance development (Sivapragasam, 1994). This poses a major problem for the implementation of the integrated pest management programme for cabbage in the lowlands (Syed *et al*, 1992).

Understanding the behaviour and ecology of *H. undalis* in the field could contribute towards the effective management of *H. undalis*. Amongst the important ecological factors that need to be investigated are that of understanding adult activity in the field. Although some information on adult activity of *H. undalis* in the field had been reported elsewhere (Yamada, 1981; Shirai and Kawamoto, 1990), such information is lacking in Malaysia. In this study, the activity of the adult male population of *H. undalis* were examined using virgin-females baited sticky traps. The latter method was used by Shirai and Kawamoto (1990) to examine flight distances of *H. undalis*. The use of virgin females is necessary as the sex pheromone, identified as an aldehyde (E11, E13)-11,13-hexadecadienal (Arai *et al*, 1982; Ando *et al.*, 1988), has not been commercially available as yet.

Materials and Methods

Adult activity on cabbage

This study was conducted in a cabbage (var. K-K cross) plot at the experimental field at MARDI, Serdang, which was surrounded by other non-cruciferous plants such as tomato, beans and fruit trees. The cabbage plot measured 12.0 m x 12.5 m with about 500 transplanted cabbage plants grown using recommended agronomic practices. Except for the preplant insecticidal spray, which was done one week before transplanting, no insecticides were used on the cabbage plants in the field.

The activity of *H. undalis* adult males in the field was monitored using a trap which had two laboratory-reared one-day old virgin females (Shirai and Kawamoto, 1990). The design of the trap was similar to the one used for trapping the diamondback moth (Reagron Pluma^{R}) (Irfan *et al*, 1991). Both the adult females were confined in a small cage measuring 10.0 cm in length and 3.5 cm in diameter constructed with 14 mesh plastic wire screen. A 10 % honey solution was provided as food for the adults. The cage was then hooked to the inside top portion of the trap. The inside bottom of the trap was covered with a thin polyethylene sheet held in place by clips and sprayed with a sticker (Kinryu^{R}, SDS Biotech). The trap was then mounted on a wooden stake and set at a height of 0.5 m above

the ground in the center of the cabbage field. The base of the wooden stake was sprayed regularly with sticker to prevent predacious insects such as ants from getting into the trap. Considering the size of the cabbage field, only one trap was used in this study. The number of *H. undalis* male moths in the trap was recorded and removed daily. The females in the small cage were replaced weekly using laboratory-reared females. Trapping was done from four days before transplanting cabbage until harvest of the cabbage. Daily records of temperature and rainfall were also made using the meteorological station at MARDI, Serdang during the duration of the experiment. To correlate trap catches with the larval population, the number of larvae on 40 cabbage plants in the same field was also counted. For sampling, the cabbage field was divided into four subplots and ten plants selected randomly in each quadrat were sampled *in situ* at 3 to 4 day intervals from 7 days after transplanting until harvest.

Daily activity pattern of adults

The daily activity pattern of the *H. undalis* males was determined at hourly intervals using one-day old virgin female moths in the trap described above. One trap was placed at four different locations in the MARDI Research Station, which also included a plot planted with cabbage. Preliminary trapping studies had shown the presence of moth in all the four locations. The experiment was repeated on five separate days which had no rain and quite similar temperature conditions. However, wind speed and direction were not recorded.

Results

Male moth catches showed random fluctuations with no apparent relationship to the larval populations in the field *(Figure 1)*. Trap catches were already high even before any *H. undalis* larvae were recorded on the cabbage, i.e. <20 days after transplanting cabbage. Similarly, at the later stage of the crop, i.e. after 40 days, adults trapped were also high. Although the local larval populations on cabbage could have contributed to the adults trapped, it does not account for the relatively high number of adults trapped after this period. In any case, larval survival to adult is generally very low (ca 1 %) for *H. undalis* under field conditions (Sivapragasam, 1994). There was also no significant relationship ($r = 0.22$; $P > 0.05$) between mean weekly trap catches at time t and the number of larvae on 10 cabbage plants at time t + 2.

Rain did not seem to influence the activity of the moths *(Figure 2)* as indicated by the low correlation ($r = 0.03$) between the number of adults caught and the incidence of rainfall.

The daily trap catches, based on the mean from four locations, suggested a bimodal activity pattern; the first and higher peak from 5.30 to 6.30 h and a relatively smaller peak at 20.30 h *(Figure 3)*.

Discussion

Trap catches of adult male *H. undalis* were higher than that expected from the field population of larvae, suggesting possible immigration of moths into the cabbage plot. The relatively high numbers of adult catches *vis-a-vis* the low larval numbers on cabbage suggested that the local population of the larvae on cabbage had little influence on the subsequent trap catches. Considering that local dispersal of *H. undalis* was common (Shirai and Kawamoto, 1990), one major source of the immigrant population on cabbage could be from its ubiquitous weed host, *Cleome rutidosperma* (D and C.). The latter is a common denizen in areas cultivated with vegetables in Serdang (Sivapragasam, 1994). One possible explanation for the fluctuating numbers trapped could be related to the age of virgin females. We found a significant correlation ($r = 0.98$; $P < 0.01$) between the age of females and the numbers trapped with fresh females generally attracting higher number of adults and their attractancy reducing with age.

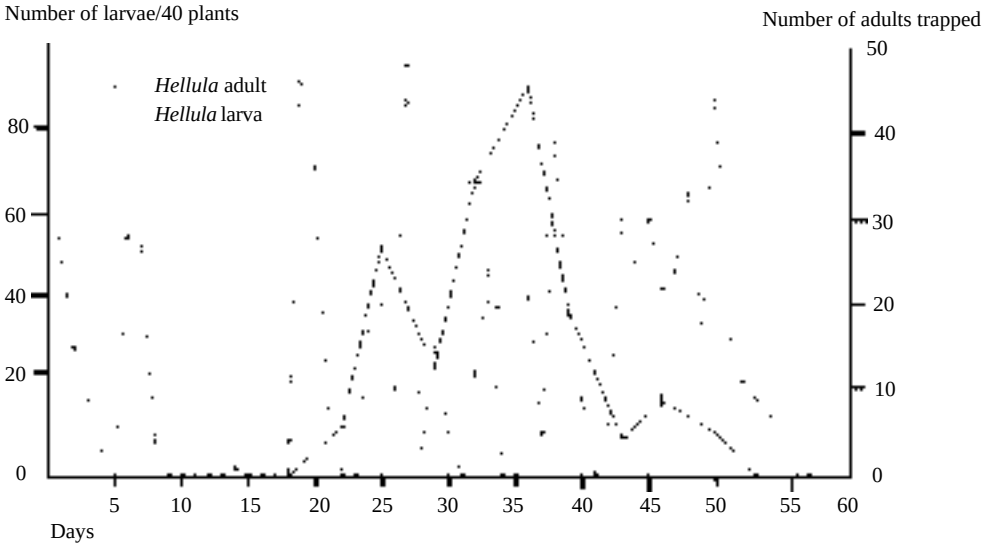


Figure 1. Trends in larval numbers and trap catches for Hellula undalis on cabbage

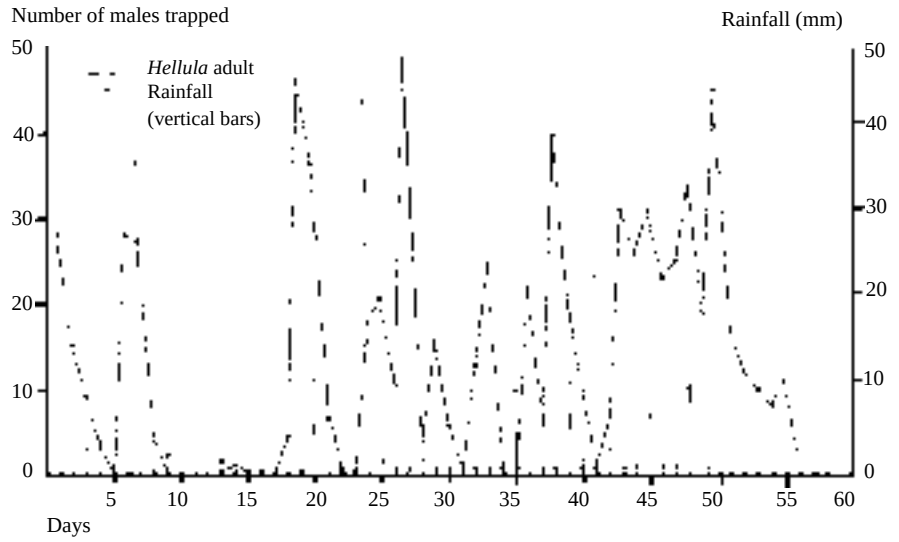


Figure 2. Fluctuations in daily adult trap catches and rainfall

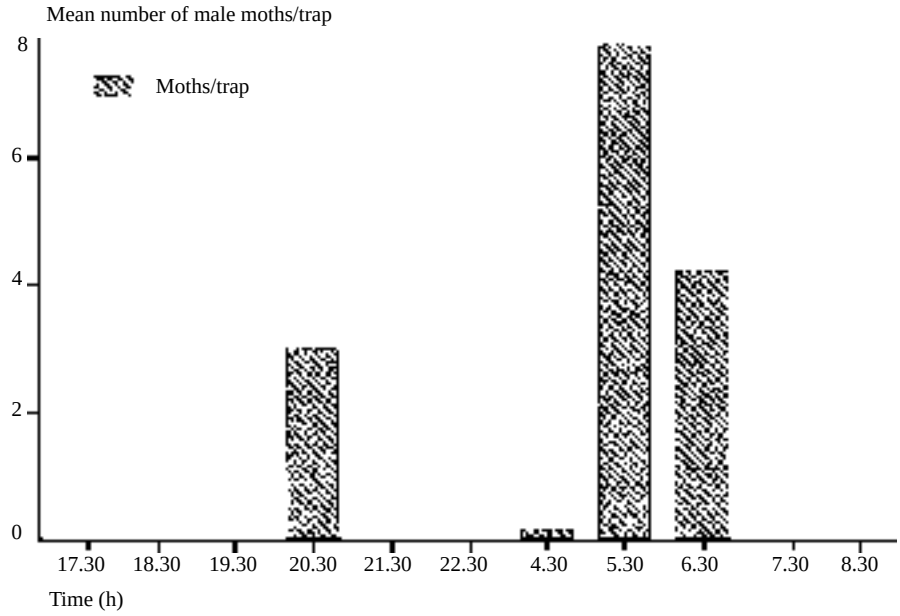


Figure 3. Hourly mean trap catches of Hellula undalis

Although rain may be important during the early larval stages of *H. undalis*, it may not be so against the other stages (Sivapragasam, 1994). Kawamoto *et al.*, (1987) however reported that temperature is more important for flight activity of *H. undalis* and the optimal temperature for this purpose is about 20 °C. However, in the Malaysian lowlands, minimum temperatures exceeded the latter value.

Talekar *et al.* (1981), also reported that in *H. undalis* adult emergence was predominantly at night. This is quite similar to that reported for the diamondback moth *Plutella xylostella* (Ashihara, 1977). But, the reason for the higher activity of the moths towards dawn needs to be investigated in the future.

Although pheromone traps have been useful to predict and time the initiation of control measures (van Steenwyk *et al*, 1983), this study suggested that the virgin female baited sticky traps per se might be limiting as a reliable predictive tool. However, in the absence of the commercial pheromones, this approach could be useful in detecting and monitoring adult populations of the moth especially in areas where crucifers are grown. Besides pheromone traps, other traps, such as the mercury vapor lamp (Sasaki, 1986) and light traps (Yamada, 1981), have also been used for trapping *H. undalis*. However, the attractancy of either sex to these traps has been reported to be very low.

References

Ando, T., Y. Ogura and M. Uchiyama (1988). Mass spectra of lepidopterous sex pheromones with a conjugated diene system. *Agric. Biol. Chem.*, ***52(6):*** 1415–1423.

Arai, K., T. Ando, A. Sakurai, H. Yamada, T. Koshihara and N. Takahashi (1982). Identification of the female sex pheromone of the cabbage webworm. *Agric. Biol. Chem.*, ***46(9):*** 2395–2397.

Ashihara, W. (1977). A laboratory bioassay method of the sex pheromone of the diamondback moth, *Plutella xylostella* (L.). *Jpn. J. Appl. Entomol. Zool.* ***(21)*** 171–172.

Irfan, U., M. Maryati, W.H. Loke and S.A. Rahman (1991). Influence of pheromone trap colour on *Diadegma semiclausum* (Horstmann). Paper presented at the International Conference on Biological Control in Tropical Agriculture. Organised by the National Coordinating Committee on Biologcal Control and Malaysian Plant Protection Society, p. 121 (abstract).

Sasaki, Y. (1986). The prevalence of occurrence and control in the summer seedling Italian broccoli with isoxanthion to the cabbage webworm, *Hellula undalis*. Bull. Kanagawa Agric. *Exp. Stn.,* ***38,*** 14–18 (in Japanese, English summary).

Shirai, Y. and K. Kawamoto (1990). A mark-recapture experiment for male adult cabbage webworm, *Hellula undalis* Fabricius (Lepidoptera: Pyralidae). *Appl. Ent. Zool.* ***25(1):*** 127–129.

Shirai, Y. and K. Kawamoto (1991). Laboratory evaluation of the flight ability of female adults of the cabbage webworm, *Hellula undalis* (Lepidoptera: Pyralidae) and reproductive success after flight. *Bull. Natl. Res. Inst. Veg. Ornam. Plants and Tea, Japan, Ser. A,* ***4:*** 31–40.

Sivapragasam, A. (1994). Biology and population dynamics of *Hellula undalis* (Fabricius)(Lepidoptera: Pyralidae) on lowland cabbages. Ph. D. Thesis, University of Malaya., 223 pp.

van Steenwyk, R.A., E.R. Oatman, N.C. Toscano and J.A. Wyman (1983). Pheromone traps to time tomato pinworm control. *California Agriculture, 37 (7/8):* 22–24.

Syed, A.R., W.H. Loke, A. Sivapragasam, I. Fauziah, U.B. Cheah, Md. M. Jusoh, A.K. Hussan and O. Yusof (1992). Integrated pest management package for effective control of *Plutella xylostella* on cabbage in Malaysia. Final report on AVNET Phase 1 for the Working Group on DBM-IPM, Lembang, Indonesia 22–26 Sept., 1992.

Talekar, N.S., W.F. Shiao and Y.H. Lin (1981). Insect pest control in summer Chinese cabbage in Taiwan. In: N.S. Talekar and T.D. Griggs (eds.): Chinese cabbage: proceedings of the First International Symposium 1981, AVRDC Shanhua, Tainan, Taiwan, China. pp 163–172.

Yamada, H. (1981). Seasonal life history of the cabbage webworm, *Hellula undalis.* Bull. Veg. Ornam. Crops. Res. Stn., A8, 131–141 (in Japanese with English summary).

Using radiation dose of 175Gy for sterile insect technique in diamondback moth

Maimun, T. & M.C. Mahani

Department of Genetics, Faculty of Life Sciences, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor.

Abstract

The suitability of using an irradiation dose of 175Gy in sterile insect technique (SIT) for genetic control of the diamondback moth was assessed by examining progeny from crosses between irradiated and unirradiated moths. Very high percentage of F_1 males carried chromosomal translocations and such markers persisted in backcross progenies. Mating success experiments showed that although spermatophore transfers were higher, irradiated moths produced fewer eggs and larvae compared to unirradiated moths. Although 175Gy is a suitable dose for inherited sterility, and to a lesser extent for SIT, low percentage of adult emergence caused by lethal mutations may hamper its practical application.

Key words: radiation dose, chromosome aberrations, mating success

Introduction

Persistence of the diamondback moth (DBM) as a pest of cruciferous crops in highland and lowland areas of Malaysia in spite of the use of chemical insecticides and biological control has led to consideration of using genetic control methods such as the sterile insect technique (SIT). For either SIT or the inherited sterility approach to control DBM, a suitable dose of radiation is required to induce mutations in release moths. The effects of gamma radiation on DBM have been recorded previously for different doses such as 100, 150, 180 and 200 Gy (Ismail & Mahani, 1993; Omar & Mahani, 1993; Ismail, 1994). These studies suggest that a dose of between 150Gy and 200Gy may be most suitable. Here we report our evaluation on the effectiveness of this dose from several aspects : chromosome mutations induced, mating success of irradiated moths, and numbers of eggs and larvae produced from the different cross combinations between irradiated and unirradiated (normal) males and females.

Materials and Methods

The DBM used in this study comprised of the Serdang strain raised on *Brassica juncea* (sawi) leaves and maintained as lab cultures for 5–10 generations, and DBM raised on artificial diet which was obtained from the insect rearing facility at MARDI Serdang. Pupae (approximately 24 hrs before emergence) were irradiated at absorbed dose of 175Gy from a Cobalt-60 Gammacell 220 source at UKM. A minimum of 500 pupae were irradiated per replication.

Emerged moths were crossed, and allowed to oviposit on sawi leaves. Fourth instar male F_1 larvae were dissected to obtain testes which was used to prepare chromosome spreads. Squash preparations stained with aceto-orcein were analysed to determine the type of chromosomal aberration carried. Fifty individuals were examined from each cross. Adult F_1 obtained from the cross between irradiated females with unirradiated males were backcrossed to unirradiated moths and chromosomal aberrations in the male progeny were similarly ascertained. In this aspect of the study only DBM raised on sawi were used.

To examine mating success, mass-reared males and females of ages 1–5 days were used in reciprocal crosses between irradiated and unirradiated individuals. After 48 hours, the females were dissected to examine for the presence of spermatophores. The number of eggs laid and larvae produced from the mating vials were also recorded.

Results and Discussion

Chromosomal translocations were detected in the majority of F1 male larvae. The average number of translocations per larva from the different crosses are shown in *Table 1*. Both chain and ring formations for multiple translocations were detected, with short chains being the most common chromosomal marker. Irradiated males x irradiated females gave F_1 with the highest number of chromosomal translocations. This result is expected as both parents carry defective gametes.

Percentage adults obtained after irradiation is generally low, with mean value of 20.91 ± 5.89 *(Table 2)*. Differences between replicates were apparently due to declining fitness of moths with increase in number of generations in culture. This was also observed for mass-reared DBM (Hussan, pers. comm.). The effect of radiation dose of 175Gy was reflected in fewer adults emerging, as well as a wide range of morphological mutants observed. These included mutations causing non-emergence of adults from irradiated pupae, such as deformed wings, stuck abdomen, stuck head and stuck wings. Many more lethalities would have been due to mutations affecting physiology, but these were not studied.

Comparison between mating success of irradiated moths with unirradiated moths show that although

Table 1. Chromosomal aberrations in male larvae from each cross

Chromosome formula	No.of reciprocal translocations	M_I x F_I	M_N x F_I	M_I x F_N	M_N x F (M_N x F_I)	F (M_N x F_I) x F_N	Total larvae with aberration type
31B or 30B + 1F or 29B + 2F	0	1	8	6	13	18	46
28B + C2	1	–	3	–	1	–	4
27B + C3	2	–	–	1	3	–	4
26B + C4	3	1	–	4	2	–	7
26B + R4	4	–	–	1	–	–	1
26B + 2 (C2)	2	1	4	3	7	1	16
25B + C5	4	2	–	2	–	–	4
25B + R5	5	–	1	–	2	–	3
25B + C2 + C3	3	4	7	3	12	3	29
25B + C2 + R3	4	–	–	1	–	–	1
24B + C6	5	1	–	–	–	–	1
24B + C2 + C4	4	3	4	1	1	2	11
24B + 2 (C3)	4	3	3	3	1	2	12
24B + C2 + R4	5	–	–	4	–	–	4
24B + 3 (C2)	3	–	–	–	1	3	4
23B + C7	6	–	–	1	–	–	1
23B + C2 + C3 + R2	5	–	–	1	–	–	1
23B + C2 + C5	5	6	1	2	2	2	13
23B + C3 + C4	5	6	5	2	2	3	18
23B + 2 (C2) + C3	4	1	3	1	1	5	11
23B + C3 + R4	6	1	–	–	–	–	1
22B + C8	7	1	–	–	–	–	1
22B + C3 + C5	6	3	2	2	–	2	9
22B + C2 + 2 (C3)	5	1	–	1	2	–	4
22B + C3 + R2 + R3	7	–	–	1	–	–	1
22B + 2 (C2) + R4	5	1	2	2	–	2	7
22B + 4 (C2)	4	1	1	–	–	1	3
22B + C2 + C6	6	1	–	–	–	–	1
22B + C4 + R4	7	–	–	1	–	–	1
21B + C4 + C5	7	3	2	–	–	–	5
21B + 3 (C3)	6	2	–	–	–	1	3
21B + C2 + C3 + C4	6	2	–	–	–	1	3
21B + 3 (C2) +R3	5	–	2	–	–	–	2
21B + C2 + C7	7	–	–	1	–	–	1
20B + 2 (C2) + 2(C3)	6	2	–	–	–	–	2
20B + C2 + C3 + C5	7	1	–	1	–	1	3
20B + 2(C3) + R4	8	–	–	1	–	–	1
20B + C2 + C4 + R4	8	–	–	1	–	–	1
20B + C10	9	–	–	1	–	–	1
19B + C2 + C4 + C5	8	2	–	–	–	–	2
19B + 2(C3) + C5	8	–	1	–	–	–	1
19B + C5 + C6	9	–	1	–	–	–	1
19B + 4(C2) + C3	6	–	–	1	–	–	1
13B + C2 + C7 + C8	14	–	–	1	–	–	1
Total larvae bearing translocations		49/50 98%	42/50 84%	44/50 88%	37/50 74%	32/50 64%	204/250 81.60%
Average translocations per larva		4.98 ± 1.06	3.48 ± 0.14	4.32 ±0.69	2.36 ± 0.84	2.52 ±0.67	17.66 ± 2.96

Table 2. Percentage of adult emergence from pupae irradiated with 175Gy

Replication	Males	Females	Total
1	20.27	21.88	42.15
2	15.90	19.23	35.13
3	7.45	8.30	15.75
4	3.25	4.92	8.17
5	4.20	3.17	7.37
6	8.88	8.00	16.88
Mean	9.99 ± 2.75	10.92 ± 3.16	20.91 ± 5.89

spermatophore transfers were higher compared to the control cross, irradiated moths suffered serious disadvantages, as number of eggs and number of larvae produced were significantly reduced *(Table 3)*. High doses of radiation have been known to cause reduction in competitiveness, low spermatophore transfer ability and reduced sexual activity (La Chance, 1985). However, SIT can be used effectively on a population that has been initially reduced by other means.

Sutrisno and Hoedaya (1992) showed that a dose of 200Gy is high enough to induce sterility, complete or partial, to the F_1 generation irrespective of the type of cross. However the use of this dose is impractical as the number of surviving progeny would be too low whereas SIT is dependent on production of large numbers of F_1 individuals carrying chromosomal aberrations. A dose of 175Gy should give higher percentage of live progeny, but would still require releases of very high numbers of pupae.

Previous study indicated that age has a significant effect on spermatophore transfer and number of larvae, with one day old females and two day old males producing most progeny (Clyde & Maimun, 1996). This is supported by results of the present study *(Tables 4 & 5)*. Analysis of variance also shows significant difference in number of progeny for interaction between crosses and males *(Table 6)*. As the cross of M_I x F_N resulted in higher number of progeny compared to M_N x F_I and M_I x F_I *(Table 3)*, this is in line with the desired objective for inherited sterility approach.

Table 3. Mean values of spermatophore transfer, eggs and larvae for four different crosses

Crosses	Spermatophore	Eggs	Larvae
M_N x F_N	0.56^b	340.71^a	182.56^a
M_N x F_I	0.76^a	40.16^c	2.09^{c*}
M_I x F_N	0.73^a	125.93^b	23.19^b
M_I x F_I	0.75^a	26.04^c	0.75^{c*}

Note: Different superscripts within a column indicate significant difference ($p < 0.05$) in Duncan's Multiple Range test.

N – Normal (without irradiation)

I – Irradiated with 175Gy

* – Zero larva more than 5%

Table 4. Mean values of spermatophore transfer, eggs and larvae for different ages of females

Age of female (day)	Sperma-tophore	Eggs	Larvae
1	0.66^c	154.48^a	69.65^a
2	0.68^{bc}	156.78^a	61.67^{ab}
3	0.70^{ab}	119.50^b	49.12^{bc}
4	0.72^{ab}	118.13^b	42.02^c
5	0.76^a	117.15^b	38.28^c

Note: Different superscripts within a column indicate significant difference ($p < 0.05$) in Duncan's Multiple Range test.

Table 5. Mean values of spermatophore transfer, eggs and larvae for different ages of male

Age of female (day)	Sperma -thophore	Eggs	Larvae
1	0.64^c	140.48^a	43.07^b
2	0.69^{bc}	137.58^a	66.02^a
3	0.66^c	142.42^a	64.07^a
4	0.74^{ab}	119.77^a	45.00^b
5	0.79^a	125.80^a	42.58^b

Note: Different superscripts within a column indicate significant difference ($p < 0.05$) in Duncan's Multiple Range test.

Table 6. ANOVA of spermatophore transfer, eggs and larvae for four different crosses

Source	DF	Spermatophore	Eggs	Larvae
Crosses	3	0.65***	1581288.20***	574840.40
Replication	2	0.01	47338.27	381290.00
Female	4	0.09	25221.76	10514.80***
Male	4	0.21***	5886.32	8391.63***
Crosses*Female	12	0.03	11160.17	5754.14***
Crosses*Male	12	0.08	4384.78	5252.39***
Female*Male	16	0.04	9212.33	1228.53
Crosses*Male*Female	48	0.03	45132.27	1142.51
Error	198	0.02	5730.00	1383.96

Note – *** very highly significant difference ($p < 0.001$)

Acknowledgement

The authors thank Mr. Hussan Abdul Kadir from MARDI, Serdang for providing mass-reared DBM. This study was funded by grant No. 57/94 from Universiti Kebangsaan Malaysia.

References

Clyde, M. M. & T. Maimun (1996). Influence of age and diet on mating success of *Plutella xylostella* (L.). XX th International Congress of Entomology, 25–31 Aug, Firenze Italy. (Abstr.)

Hussan, A. K. (1996). Personal communication. Basic Research Division, MARDI, Malaysia.

Ismail, B. (1994). Radiation-induced changes in reproductive ability of Diamondback Moth (Lepidoptera: Plutellidae). Journal of Economic Entomology 87: 1190–1197.

Ismail, B and M. Mahani (1993). Radiation-induced genetic damage in *Plutella xylostella.* Jurnal Sains Nuklear Malaysia 11: 69–76.

LA Chance, L.E. (1985). Genetics methods for the control of Lepidoptera spesies: Status and potential. U. S. Dept. of Agric, Agricultural Res. Service ARS 28–40 pages.

Mahani, M. and B. Ismail (1995). Chromosome markers for sterile insect technique (SIT) in *Plutella xylostella* L. National Seminar on Entomology, 18–19 March, Universiti Malaya Kuala Lumpur. (Abstr.)

Omar, D. and M. Mahani (1993). Effect of substerilization doses of radiation on the biology of Diamondback Moth. Pp 3–9 *In*: Radiation Induced F1 Sterility in Lepidoptera For Area-Wide Control. IAEA Vienna.

Sutrisno, S. and M. Hoedaya (1992). The sterile insect technique and transmission of inherited sterility to control the Diamondback Moth, *Plutella xylostella* (L.), and the Cabbage Webworm, *Crocidolomia binotalis* Zell. Pp 395–403 *In* : Management of Insect pests : Nuclear and Related Molecular and Genetic Technologies. IAEA, Vienna.

Mass rearing of diamondback moth on artificial diet: potential for parasitoid production and population studies

Hussan A.K., A. Sivapragasam, A. Asma and R. Azman
Strategic, Environment and Natural Resources Research Center,
MARDI, Serdang, P.O. Box 12301 50774 Kuala Lumpur, Malaysia

Abstract

The method of rearing *Cotesia plutellae* (Kurdj.) and *Oomyzus sokolowskii* using diamondback moth reared on artificial diet and the method of marking DBM adults using calco oil red dye (337.5 mg per 0.5 gallon of diet) for use in population studies are described.

For *C. plutellae*, our studies showed that the mean number of cocoons produced per cup was about 89% based on initial larval density (range 60–100). Mean developmental period was 14.8 days, slightly longer than reported elsewhere. This was, however, offset by the larger adult size. The sex ratio of females to males was 0.57:1.

For *O. sokolowskii*, parasitization per cup was 70–90% based on initial pupal density. The parasitoids emerged from the pupa in 15–19 days after initial exposure to the parasitoids. Mean number of parasitoid emergence per host pupa was 26 (range: 6–42). Parasitised pupae may be stored at 5–6 °C for up to 2 months.

Comparison of DBM reared on diet with and without dye based on number of pupa formed, adult emergence, developmental time, sex ratio, eggs per female and adult longevity, respectively, showed no significant differences. The potential of using artificial diet for the production of the parasitoids as well as for marking adults for population studies (e.g. local dispersal) are discussed.

Key words: diamondback moth, parasitoids, artificial diet, adult marking

Introduction

The mass rearing of crucifer pests in MARDI is presently being carried out using artificial diet. One of the pests being reared is the diamondback moth (DBM), *Plutella xylostella*. The rearing of insects on artificial diet is a convenient method as it is capable of satisfying some of the research needs, ie. it affords reliability and efficiency in production. Rearing using artificial diet is an expensive affair and once committed the cost is incurred whether the insects are being used or not. That being the case, the utilization of insectary facilities where possible should be optimized. In the insectary at MARDI, with DBM, there is always a surplus of eggs which could be utilized beneficially for parasitoid production. The DBM being reared could also be utilized for carrying out population studies if the insect could be suitably marked. This paper presents preliminary results on the rearing of two major parasitoids of DBM; *Cotesia plutellae* (Kurdj.) and *Oomyzus sokolowskii* (Kurdj.) and also results of a marking study on adults of DBM using the calco red dye incorporated into the diet.

Materials and Methods

Rearing of Cotesia plutellae

The rearing procedures for the *P.xylostella* host larvae were as described in Hussan and Sivapragasam (1996). Two 2-day old mated female *C. plutellae* were introduced for 24 hours in cups containing 90–100, 2nd instar (6–7 day old) DBM larvae. After 24 hours, the adults were removed and the parasitised DBM larvae were reared at 25ºC and 70–80 % relative humidity until emergence of the parasitoids. After 7 days, the diet cake was removed to reduce fungal contamination.

Rearing of Oomyzus sokolowskii

The host DBM larvae were reared on the artificial diet mentioned above. One hundred parasitoids (approximately 50 pairs) were introduced into 8 ozs (about 240g) cups containing approximately one hundred 4th instar larvae. The adult parasitoids were fed with honey solution. Parasitoids were allowed to parasitize for 3 days. Upon pupation, the pupa were kept further for several days to allow adult DBM to emerge from the unparasitized pupae. Pupae containing the parasitoids were then separated.

Marking studies

The feasibility of using calco red dye for marking DBM adults was evaluated by comparing the development of DBM on marked and unmarked diet. For 1/2 gallon (@2.27 litres) of diet, 337.5 mg of calco red dye was added (Hussan and Sivapragasam, 1996). Approximately 25 ml of the diet was poured into 8 oz (about 240 g) styrofoam cups. Approximately 100 eggs were introduced into each cup and covered. Rearing was carried out at 25ºC and 80% relative humidity.

Table 1. Development of DBM on diet containing calco red dye

Parameters	Treatments[1] +dye	-dye	t-test
No. of pupae (mean/cup)	45.4 (10)	51.5 (12)	NS
No. of adults (mean/cup)	36.5 (10)	33.1 (12)	NS
Total development time (egg to adult emergence, days)	17.8 (49)	16.3 (28)	P<0.05
Sex ratio of adult (Female to male)	0.49	0.44	NS
Egg per female (Mean)	108.5	100.2	NS
Longevity of adults			
male (n = 30)	7.1	6.6	
female (n = 30)	8.3	7.6	

[1]Values in parentheses indicate numbers of samples used. NS – not significant at P=0.05 using the paired t-test

Results and Discussions

Rearing of parasitoids

For *C. plutellae*, our studies showed that the mean number of cocoon produced per cup was about 89% (Range: 60–100) based on initial density. Mean developmental time was 14.8 days, slightly longer than reported elsewhere using other food sustrates (Lim and Chan, 1986). However, larger adults were obtained using the larvae from the diet. The sex ratio of females to males was 0.57:1.

For *O. sokolowskii*, parasitization per cup was 70–90% based on initial pupal density. The parasitoids emerged from the pupa from 15–19 days after initial exposure to the parasitoids. Mean number of parasitoid emergence per host was 26 (Range: 6–42). Parasitized pupae may be stored at 5–6 ^{o}C for 2 months.

Marking studies

The results of the marking studies using calco red dye showed no significant difference between DBM reared on diet with and without the dye *(Table 1)*. The red colour was clearly visible for the larval and pupal stages but not for the adult stage. Marked adults could however be easily identified by teasing and examining fat tissues. Initial studies showed that the incorporation of the calco red dye is suitable way to mark large number of DBM larvae especially for population and dispersal studies in the field (Jusoh Mamat, pers. comm.).

Discussion

In the lowlands of Malaysia, the growing of crucifers like cabbages, are usually not continuous. This being the case, the production and timely release of parasitoids becomes important because they play a central role in IPM programmes for DBM (Lim and Chan, 1986). Netted houses are also increasing in popularity and the use of parasitoids should auger well with cultivation under net. Our studies have shown the ease and feasibility of using artificial diet for rearing host larvae for parasitoid production. The capability to mass produce parasitoids should facilitate studies such as to evaluate the effectiveness of the parasitoids in a field situation.

The rearing of insects using artificial diet is easier and more efficient compared to using the natural host plant. However, contamination of the diet by microorganisms are a constant threat. Strict hygiene is absolutely necessary to prevent disruption in rearing.

References

Hussan A. K. and A. Sivapragasam (1996). Mass rearing of lepidopterous insect pests of crucifers Proceedings Asian vegetable Network II Final Workshop, 1–6 September 1996, Bangkok, Thailand.

Lim, G.S. and and Chan, H.F. (1986). A simple rearing unit for the diamondback moth and its parasitoid *Apanteles(=Cotesia) plutellae* Kurdj. *MARDI Res. Bull.* ***14****:* 154–157.

Workshop Summary

Denis J. Wright

Department of Biology, Imperial College of Science, Technology & Medicine, London University, Silwood Park, Ascot, Berkshire SL5 7PY, U.K.

Over the past four days, we have heard and seen a wide variety of excellent talks and posters on the applied biology of the diamondback moth (DBM) and a number of other crucifer pests. All of the participants and especially the members of the organising committee from MARDI and MAPPS should be congratulated on what has been a highly successful and very enjoyable meeting with unparalleled hospitality. It is not possible to mention specifically all of the work presented in the time available but I think we would agree that each of the four lead speakers made a particularly stimulating and original contribution to the meeting.

A survey of the topics covered reveals that one third were concerned with chemical control, with insecticide resistance alone accounting for about 25% of the papers and posters presented. This reflects the central importance that the exceptional ability of DBM to evolve resistance still has on its status as a major pest species. With increasing reports of resistance to *Bacillus thuringiensis*-based products in populations from Asia and the Pacific to the Americas, this subject area is likely to remain extremely topical. Populations of DBM provide, for example, the only field model at present for *B.t.* resistance. Richard Roush, in his paper, advocated the need to consider radical approaches to the management of DBM and such insecticide resistance problems. In an excellent talk, he stressed the particular value that transgenic plants with multiple B.t. toxins "pyramided' within the same variety could have if used within an integrated resistance management (IRM) programme.

Peter Ooi, in his paper, outlined very clearly the interrelated problems of overuse of pesticides, the development of insecticide resistance, increasing pesticide residues and the destruction of natural enemies, that have resulted in a spiralling escalation towards crop disaster in some areas, and as Peter Ooi so memorably put it, the final, IPM phase! It is perhaps not suprising therefore that over 50% of the papers and posters presented at this meeting were devoted to alternative, non-chemical methods of control and their inclusion within IPM programmes. In practice, these are ICM programmes, since crop management strategies are of central importance for integrated control. As we have seen this week, the host crop can have various (multitrophic) interactions with pest and its natural enemies, and also influence the bioavailability of insecticides.

Biologically-based approaches to the control of DBM were reviewed thoroughly in the paper by Anthony Shelton, co-authored by Perez, Tang and Vandenberg, who in a wide-ranging talk discussed how various control methods, including the use of partial host plant resistance, pheromones, baculoviruses, parasitoids and entomopathogens, could be used in conjunction with the more selective application of pesticides to minimise the negative aspects of chemical control.

The dynamic nature of insect and other pest problems on crucifers was highlighted in the paper presented by Lim Guan Soon, co-authored by Sivapragasam and Loke Wai Hong, who pointed out the complex and various interactions between DBM and other pest species, some of which have been relatively poorly investigated. A striking example of a relatively new problem, discussed in a subsequent paper, is the emergence of snails as widespread pests of crucifers in the Cameron Highlands of Malaysia.

Lim Guan Soon, Peter Ooi and a number of other participants also emphasized the need for continued improvements in the implementation of integrated management programmes. Particular problems include weak extension support and the continuing gap between research and farmer's needs. The importance of season-long training of "trainers'" and of farmer field schools in the successful implementation of IPM programmes was emphasized in relation to experiences in several South East Asian countries. This included the need for farmers to recognise the importance of reducing pesticide pressure in order to conserve effective natural enemies such as the parasitoid *Diadegma semiclausum*.

It is clear from what we have heard and seen this week, that the key components of IPM or ICM programmes can vary depending upon the nature of the agroecosystem involved and, in particular, on the average farm size, the diversity of the crop production system and the economic value of the crucifer crop. In all cases, however, the importance of educating farmers, suppliers and consumers is clear, as is the need for appropriate methods of enforcement and the provision of realistic alternatives to chemical control.

In conclusion, I would like to thank again on your behalf, all our Malaysian hosts, including the Minister of Agriculture, Datuk Amar Dr. Sulaiman bin Haji Daud for officiating the opening of this workshop, and Tuan Hj. Embi Yusoff, Deputy Director General of MARDI for presiding over the closing ceremony. This meeting has been a worthy successor to the DBM Workshops in Taiwan in 1985 and 1990, organised so well by N.S. Talekar, and I look forward to the next workshop, to be held in Australia in four years time.

Index

(unless species name stated otherwise, all index words apply to diamondback moth, *Plutella xylostella*)