PROCEEDINGS

The Sixth International Workshop on **Management of the Diamondback Moth and Other Crucifer Insect Pests**

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Editors

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AVRDC – The World Vegetable Center ▪ Cornell University ▪ Kasetsart University

AVRDC – The World Vegetable Center is an international nonprofit research institute committed to alleviating poverty and malnutrition in the developing world through the increased production and consumption of nutritious and health-promoting vegetables.

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Foreword

Vegetable brassicas such as cabbage, cauliflower, broccoli, mustard, radish, and several leafy greens are important crops worldwide, with an acreage of 2.29 million ha. Asia alone accounts for more than 70% of global brassica acreage as well as production. Brassicas contribute essential vitamins and minerals, especially vitamins A and C, iron, calcium, folic acid, and dietary fiber to the human diet, and also contain several compounds that prevent or protect against different types of cancer.

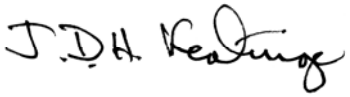
Diamondback moth (DBM), *Plutella xylostella*, is the most serious pest worldwide of all economically important brassicas. In addition to DBM, secondary pests such as head caterpillar (*Crocidolomia binotalis*), web worm (*Hellula undalis*), butterfly (*Pieris* spp.), flea beetle (*Phyllotreta* spp.) and aphids (*Brevicoryne brassicae, Lipaphis erysimi, Myzus persicae*) also cause significant yield losses. Farmers prefer to use chemical pesticides for controlling these pests because chemicals have an immediate knock-down effect, and are readily available in local markets. DBM management costs up to US$ 1 billion per year globally. A recent estimate showed that in India alone, DBM control costs about US$ 168 million per annum. Control measures constitute about 38% of the cost of production of major brassica crops in India and about 49% in the Philippines. Many pesticides used in brassica production systems are highly toxic to human health. For example, one popular pesticide for cabbage crops in Thailand is *methyl parathion*, an organophosphate. This product has been banned by most countries, and is classified by World Health Organization (WHO) as a Class 1a (extremely hazardous) substance. Similarly, at least ten of the active ingredients used in brassica production systems in parts of India were listed as extremely or highly hazardous (classes Ia and Ib) by the WHO. Pesticide abuse in brassica production poses serious risks to producers, consumers, and the overall health of the environment, and has triggered a growing interest in integrated pest management (IPM) techniques.

AVRDC – The World Vegetable Center took the lead in IPM for DBM in Asia. The Center implemented a brassica IPM program under the Asian Vegetable Network (AVNET) from 1989-1996. It introduced parasitoids such as *Diadegma semiclausum*, *Cotesia plutellae*, *Diadromus collaris,* and *Trichogrammatoidea bactrae* in Indonesia, Malaysia, the Philippines, and Thailand. The biopesticide *Bacillus thuringiensis* complemented the action of these parasitoids. Participating farmers from collaborating countries adopted IPM, resulting in a significant reduction in pesticide use that drastically reduced the cost of production and enhanced environmental health. Similar biological control programs were implemented in Cambodia, Laos, and Vietnam (CLVNET) from 1996-2005, in South Asia (SAVERNET) during 1992-2000 and in East Africa during 2004-2008. Most other organizations such as the Food and Agriculture Organization, *icipe* – African Insect Science for Food and Health, International Institute of Tropical Agriculture, national agricultural research services and universities are leading similar programs in different parts of the world. Currently, secondary insect pests of brassicas receive much less research attention than DBM—an imbalance that should be addressed in future research activities.

The International Working Group on DBM and Other Crucifer Insects is an informal group of researchers worldwide who are actively engaged in research and development in brassica pest management. Every four to five years the group organizes and participates in an international workshop on the management of DBM and other crucifer insect pests. I am happy to note that

AVRDC – The World Vegetable Center is an active member of this working group. The Center hosted the first and second workshops in Taiwan in 1985 and 1990, respectively. In collaboration with Cornell University (USA) and Kasetsart University (Thailand), the Center organized the *Sixth International Workshop on Management of the Diamondback Moth and Other Crucifer Insect Pests* at Kasetsart University's Kamphaeng Saen campus, Nakhon Pathom, Thailand from March 21-25, 2011. About 120 participants from 22 countries participated in the workshop.

The *Proceedings* reflects recent research and development issues in the management of DBM and other insect pests on brassicas. It summarizes recent advances in the fields of bio-ecology and behavior of DBM and other crucifer pests, host plant resistance, biological and non-chemical management, insecticides, IPM systems, genomic and other novel approaches, and also explores global challenges in the 21st century in brassica pest management. I trust this *Proceedings* will serve as a resource for researchers, extension staff, entrepreneurs, and policymakers in research, education, and the agrochemical sector.



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Acknowledgements

The Sixth International Workshop on Management of the Diamondback Moth and Other Crucifer Insect Pests was organized by AVRDC – The World Vegetable Center in collaboration with Cornell University (USA) and Kasetsart University (Thailand). The workshop was held at Kasetsart University's Kamphaeng Saen campus, Nakhon Pathom, Thailand from March 21-25, 2011. Forty-eight oral presentations and 19 poster presentations were made in seven scientific sessions: diamondback moth and other crucifer insect pests - global challenges in the 21st century; biology, ecology and behavior of diamondback moth and other crucifer pests; Insect - plant interactions, chemical ecology and plant resistance; biological and non-chemical management of crucifer insects; insecticides and insecticide resistance; overcoming barriers to development and implementation of IPM systems for crucifers; and genomic and other novel approaches to crucifer pest management. I thank all 114 delegates from 22 countries whose participation made this workshop a great success.

I would like to acknowledge Dr. Jacqueline d'Arros Hughes (Deputy Director General for Research, AVRDC – The World Vegetable Center, Taiwan) and Dr. Sombat Chinawong (Vice-President, Kamphaeng Saen campus, Kasetsart University, Thailand), who served as the Workshop Presidents and provided all necessary support. I also thank the Organizing Committee members, especially Dr. Anthony M Shelton, Dr. Brigitte Nyambo, Dr. David Grzywacz, Dr. Myron Zalucki, Dr. Rami Kfir, Dr. Sivapragasam Annamalai, and Dr. Zhenyu Li who assisted me in developing the scientific program. I am equally grateful to Dr. Sermsiri Chanprem, Dr. Sirikul Wasee, Dr. Thammasak Thongket and Ms. Kanokwan Laoaroon of Kasetsart University, and Dr. Robert Holmer and Mr. Steve Kebasen of AVRDC East and Southeast Asia who provided great support and excellent logistics for a successful workshop.

I am very grateful to Kasetsart University for the conference venue. Generous financial support from the Ministry of Foreign Affairs (MOFA) of Republic of China (Taiwan), DuPont, Dow AgroSciences, Valent BioSciences Corporation and East-West Seed helped us to bring in invited speakers and eminent *Brassica* IPM researchers from around the world.

I also thank Dr. Anthony M Shelton, Ms. Hilda L Collins of Cornell University, and Ms. Maureen Mecozzi of AVRDC – The World Vegetable Center for editing the text, and Ms. Chen Te-ying (Kathy) and Ms. Chen Mei-hong (Angela) of AVRDC for formatting the Proceedings. Finally, thanks to OMNIPRESS (Madison, WI, USA) for setting up the abstract and final papers collection system, and preparing the Proceedings CD.

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SESSION 1

Diamondback moth & other crucifer insect pests - global challenges in the 21st century

Behavioral and genetic components of a host range expansion in the diamondback moth

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ABSTRACT
The diamondback moth (*Plutella xylostella*, Lepidoptera: Plutellidae) is a specialist on Brassicaceae and besides being a major pest insect, is often studied in its relationship with these plants and their specific chemistry. It was therefore unexpected to find this insect infesting sugar snap peas (*Pisum sativum*, Fabaceae) in Kenya in 1999. Colonies originating from that area in Kenya that are reared separately on both cabbage (*Brassica oleracea* var. *Gloria*) and pea were used to study the behavioral and genetic mechanisms behind this apparent host range expansion. Neonates of both strains are initially attracted to leaf discs of both cabbage and pea, but the cabbage strain prefers cabbage if given more time to make a choice. Choice after a period of 24 h is indicative of whether the insect can survive on a given host plant. Assays testing whether neonates leave a leaf disc on which they were placed show that cabbage strain neonates are significantly more likely to reject pea than are neonates of the pea strain. Neonate acceptance of a host is correlated to ability to complete development on it and is therefore an important component in this host range expansion. The genetics and relationship of survival and relevant behavioral traits are discussed. Ability to complete development on pea appears to be partially dominant, but neonate choice and acceptance show intermediate frequencies in F1 hybrids. There are thus likely to be additional traits with a genetic basis responsible for successful utilization of this novel host.

Keywords
Behavior, host shift

INTRODUCTION
Agricultural ecosystems provide opportunities to directly test and apply evolutionary concepts, and likewise, evolutionary theory informs agricultural management decisions. For the study of evolutionary or ecological principles, agroecosystems have the advantage over non-managed systems, in that much of the recent natural history of the environment is known or can be ascertained more easily. In this way, we may directly test the relative contributions of standing genetic diversity, effective population size, density dependence, or other factors.

Conversely, integrated pest management is also guided by evolutionary and ecological principles. Insect resistance management (IRM) is the delay or prevention of evolution of resistance in insect pest organisms. A common IRM strategy in cropping systems is the use of an untreated refuge as a source for susceptible alleles in the pest population (Bates et al. 2005; Gould 2000) to counter the selection for resistance alleles. Thus, success of the refuge strategy is dependent on sufficient movement between treated and refuge areas so that resistant survivors mate with unselected susceptible individuals. Probability of movement is determined firstly by the insect's mobility, but also by behavioral traits influencing whether the insect can distinguish between treated and refuge plants (Shelton et al. 2000; Tang et al. 2001). Refuge plants, however, need not necessarily be the same species as the protected plants, so alternative hosts may be an option (Onstad 2008; Jongsma et al. 2010).

In a similar vein, the extent of utilization of alternative hosts influences the success of pest management practices. Trap cropping is a cultural control method that involves the sacrificial planting of an attractive low-value crop as a sink for pest insects to protect the high-value crop. Deployment of particular strategies depends on the life history of the pest insect, and for diamondback moth, the trap crop is used to attract egg-laying females to plants on which the larvae have low survival (Shelton and Badenes-Perez 2006). Yellow rocket, *Barbarea vulgaris*, shows promise to be used as a trap crop for diamondback moth on cultivated crucifers, in that females prefer *B. vulgaris* for egg-laying, but larvae show little to no survival on the plants (Badenes-Perez et al. 2004, 2005). To determine the sustainability of this dead-end crop to reduce populations, it is therefore necessary to understand behavioral factors influencing larval survival and also the range of behavior among diamondback moth populations.

One aim of this study is to examine general mechanisms of how neonate larvae select and accept host plants. Neonate larvae are used because that is the most biologically relevant immature life stage; once larvae accept a host plant, they will remain on it unless available plant material is diminished. The diamondback moth is well-known for its association with *Brassica* crop plants (Talekar and Shelton 1993), although it has also been reported to be able to survive on alternate hosts

(Gupta and Thorsteinson 1960; reviewed in Sarfraz et al. 2006).

The diamondback moth was discovered on sugar snap peas (*Pisum sativum*, Fabaceae) in the Naivasha region of Kenya in 1999 (Loehr 2001). The strain collected from this infestation (DBM-P) is able to complete development on both pea plants and the ancestral *Brassica* plants, indicating that a host range expansion rather than a host shift has occurred (Loehr and Gathu 2002). Adult females of the pea strain retain a preference to lay eggs on cruciferous plants, but they are more likely than other strains to lay eggs on pea plants in choice and no-choice experiments (Henniges-Janssen et al. 2011).

Loehr and Gathu (2002) proposed that survival on pea was related to "the ability to initiate feeding without the normal stimuli present in crucifers." Subramanian and Loehr (2006) conducted an experiment of host plant choice of neonates, and we would like to expand from this work. Thus, the additional aims of this study are to better characterize the behavioral components contributing to the host range expansion in the pea strain and determine the inheritance of those components.

MATERIALS AND METHODS

Petri dish assays

Observational assays of a duration of 5 minutes were conducted to measure attraction to the two different host plants in choice and no-choice scenarios. Observations were made every 30 seconds and included whether a neonate reached a particular leaf disc and whether a neonate left its respective sector (Figure 1). In this way, host searching behavior can be characterized by both speed and level of directed movement.

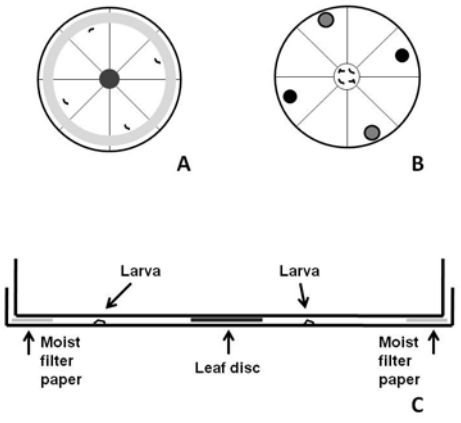

Figure 1. Diagram of the petri dish assays (not necessarily to scale). A) No choice assay; B) Choice assay; C) Profile view of the no choice assay.

No choice

The arena consisted of an inverted lid, into which was placed the bottom of the petri dish (Figure 1A), leaving about 1-2mm vertical space in which the neonates could walk (Figure 1C). This was done because some neonates prefer to hold an inverted orientation, and the small vertical space allowed neonates to do so. In the center of the arena was placed a leaf disc (14mm) of either cabbage or pea, and a ring of moist filter paper (width 1cm) was placed around the outer edge both for moisture and as a deterrent to neonate escape. Neonates were placed (15mm) away from the edge of the arena and were observed four at a time per family.

Choice

The arena was set up similarly to the no-choice experiments, but with neonates beginning the assay inside a 15 mm circle in the center and with leaf discs placed 3mm from the edge (Figure 1B). Two cabbage leaf discs and two pea leaf discs (12mm) were placed opposite each other, respectively. Two or four neonates per family were observed at a time; observations are the same as described above. Most neonates made a choice within the 5 minute assay; the 7 of 76 total neonates that did not make a choice were excluded from analyses. In these choice assays, the petri dish arenas were checked 24 h after the initial observation to see whether the proportion of neonates on the respective leaf discs was the same.

Vial assay

Based on results from the short term petri dish assays, an assay of longer duration on individual neonates was devised. Plastic vials with a height of 2.5 cm and width of 1.2 cm contained a leaf disc of cabbage and pea at either side. Leaf discs were randomized with regard to position in the vial. Neonates were placed on the side of the vial between the leaf discs and were checked 24 h later for their choice. Vials were placed on their side for the assay so that preferred orientation of neonates was not a confounding factor in the experiment.

To test applicability of this assay for use as a marker, results were compared to survival data in two ways. First, the percentage of neonates per family that chose the pea disc was compared to the percent of individuals per family that were able to complete development to pupation on a diet of pea leaves. Individuals were siblings and not the same ones used to test survival. In a separate set of replicates, however, larvae were reared to pupation on their choice as neonates.

RESULTS AND DISCUSSION

Petri dish assays

No choice

There was no difference in attractiveness of the two host plants between the two host races. Equal proportions of the cabbage strain and the pea strain go to both the cabbage (χ^2=0.066, *P*=0.797, n=52) and pea leaf discs (χ^2=0.265, *P*=0.607, n=52) in the 5 minute assay (Figure 2). In a test for level of directed movement, *i.e.* how many neonates left their sector, the pea strain exhibited

more wandering behavior in the presence of pea compared to cabbage (χ^2=6.38, *P*=0.012). There was no difference in directed movement of cabbage strain neonates in assays with cabbage or pea leaf discs (χ^2=0.018, *P*=0.894).

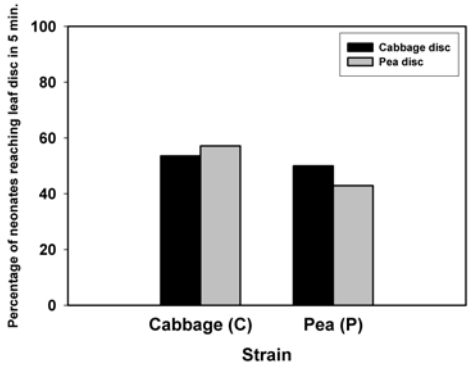

Figure 2. Proportion of neonates of each strain that reached a leaf disc within the allotted 5 minute no-choice assay. There was no significant difference between or among the strains with respect to host plant in a Chi square test (n=52).

Choice

There was no association between larval strain and the leaf disc they chose (n=62; χ^2=0.132; *P*=0.717), meaning that neonates of both strains go to both leaf discs in the 5 minute assay (Figure 3). For level of directed movement, there was no difference between the strains for either chosen disc (cabbage χ^2=0.305, *P*=0.581; pea χ^2=1.23, *P*=0.268).

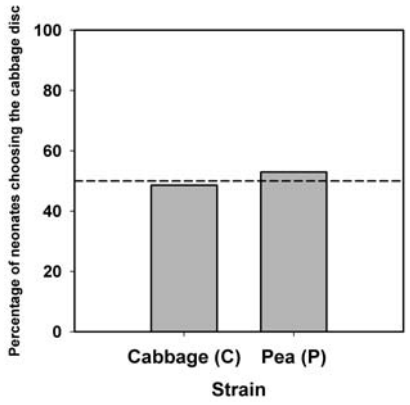

Figure 3. Proportion of neonates choosing a cabbage leaf disc within the allotted 5 minute choice assay; the proportion choosing pea is the inverse of those choosing cabbage. Of the neonates making a choice (69 of 76), there was no significant difference in a Chi square test of independence between the strains in which host plant they chose.

In the short-term five minute assays, neonates of both the cabbage strain and the pea strain went to leaf discs of both host plants. In the choice test, however, when the arenas were checked 24 h later, the distribution of cabbage strain neonates on the leaf discs had changed. Even though 60% of arenas had an equal or majority number of cabbage strain neonates on pea compared to cabbage after the five minute assay, only 30% of the total replicates showed this pattern after 24 h (Figure 4). The distribution of pea strain neonates remained the same from the 5 minute recording to 24 h later, suggesting that pea strain neonates remain with their original short term choice. One other possible explanation for the observed distributions, that pea strain neonates periodically sample leaf material, was not evident from multiple feeding sites on the leaf discs, nor does it reconcile with the typically observed mining behavior. That cabbage strain neonates went to leaf discs of both plants in the short term, but not 24 h, suggests that perhaps detection of general green leaf volatiles or even humidity plays a role in initial attraction to a host plant.

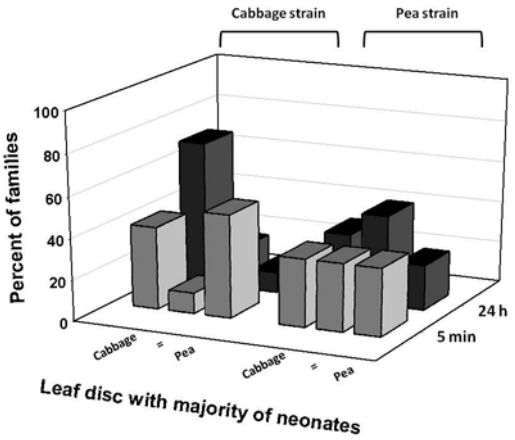

Figure 4. Distribution of neonates in the choice assay at 5 minutes compared to 24 h later (n=19 families). Cabbage strain neonates initially go to leaf discs of either cabbage or pea in the 5 minute duration, but there is a preference for cabbage leaf discs at the 24 h time period. Pea strain neonates tend to remain with their original choice.

Vial Assay

In a test for equal proportions the cabbage strain preferred the cabbage leaf disc (n=71, χ^2=31.11, *P*<0.0001), and the pea strain preferred the pea leaf disc (n=61, χ^2=4.738, *P*=0.030; Figure 5). For the F1 hybrids, offspring of a cabbage strain mother and pea strain father showed no preference for either leaf disc (n=48, χ^2=0.083, *P*=0.773). There was a marginal preference of offspring from a pea strain mother and a cabbage strain father for the cabbage leaf disc (n=125, χ^2=3.528, *P*=0.060).

To test applicability for use as a marker, the percentage of neonates choosing the pea disc in the vial assay was compared to the percentage of its siblings that were able to complete development on pea. There was a significant correlation between choice of pea and survivorship on pea among the 35 families of both strains and also F1 offspring (r=0.494, *P*=0.003; Figure 6). A separate set of replicates was used to measure the survival to pupation on whichever host plant a given neonate had chosen.

There was no difference in survival between whether a neonate chose cabbage or pea (χ^2=0.28, df 29, *P*=0.595; Figure 7).

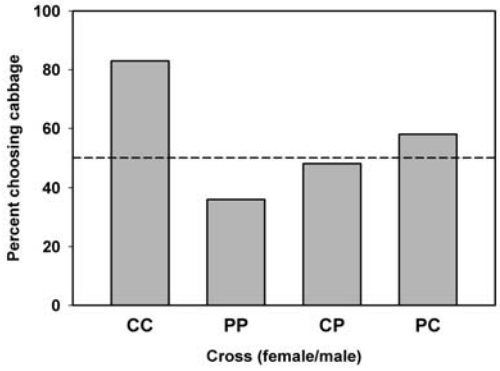

Figure 5. Proportion of neonates selecting the cabbage disc in the 24 h vial assay; the proportion choosing pea is equal to the inverse of those choosing cabbage. The parental cabbage and pea strains significantly chose both cabbage and pea, respectively, in a Chi square test of equal proportions. The F1 hybrids in crosses of both directions did not show a significant preference, although offspring of a pea strain mother and cabbage strain father showed a slight, marginally significant preference for cabbage (*P*=0.06).

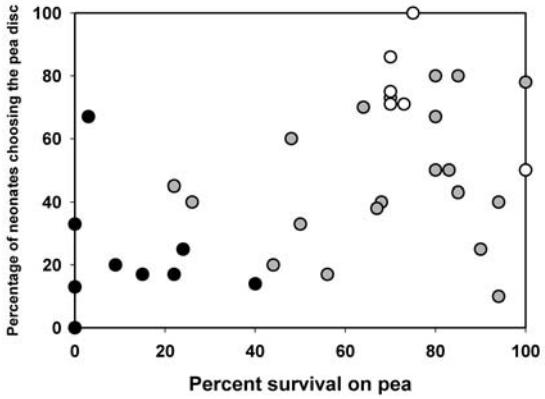

Figure 6. Positive relationship between percent of neonates choosing the pea disc in the 24 h vial assay and percent survival of their siblings (r=0.494; *P*=0.003). Parental cabbage strain families are denoted with black circles, parental pea strain families are denoted with open circles, and F_1 hybrid families are denoted with gray circles.

The 24 h vial assay has potential for use as a discriminating marker trait. Most importantly, individual neonate choice in the vial assay is reflective of their ability and their siblings' ability to survive on pea. However, one might expect, based on the results of the petri dish assay that pea strain neonates would show no preference, but this effect was not observed. The percentage of both strains choosing pea is higher than that reported in Subramanian and Loehr (2006), but their assay design tests multiple neonates in fewer replicates in a petri dish arena.

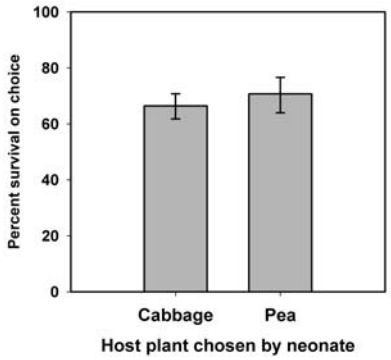

Figure 7. Percent survival to pupation on whichever host the larvae chose as a neonate in the 24 h vial assay. Results are pooled by chosen host plant; there is no difference in survival between cabbage and pea (*P*=0.595).

Comparing the assays

Gustatory or physical cues may then influence whether a neonate remains on the host plant and initiates feeding. Loehr and Gathu (2002) suggest that the adaptation to pea is largely dependent on whether the neonates will mine on pea leaves. Acceptance of alternate hosts can be enhanced by the application of sinigrin to leaves (Gupta and Thorsteinson 1960); in fact, incorporation of sinigrin and other crucifer compounds makes artificial media palatable to diamondback moth larvae (Thorsteinson 1953). Sinigrin application to pea leaf discs has been shown to increase its palatability to older larvae (van Loon et al. 2002; Gupta and Thorsteinson 1960), and while it has not yet been tested on neonate larvae, one would expect the same effect.

While there does appear to be genetic control of choice of host plant by neonates, the vial assay could be altered in a way that promotes a clearer separation of phenotypes. One aspect to consider is the likelihood of a larva to be able to make a choice in the field, given their limited mobility. One example of a behavioral assay that may be more reflective of nature is a "passing-over test", where neonates are examined for whether they continue searching for a host after passing over another host (Stuhl et al. 2008). An assay testing whether naïve neonates will reject a pea leaf disc is currently underway.

CONCLUSION

Changes in larval behavior can be subtle to detect, but clearly there was enough variation in the field to produce noticeable effects of damage on peas from herbivory by diamondback moth larvae (Loehr 2001). The results from experiments on neonate choice demonstrate the importance of the scale on which the plant cues affect behavior. Neonates initially went to leaf discs of the host plants, but physical or gustatory mechanisms likely

influenced whether larvae remained with a particular host plant. Neonate acceptance of a particular host plant appears to be under genetic control and is one important component contributing to the ability to complete development on the novel host.

Acknowledgements

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Predicting outbreaks of a migratory pest: an analysis of DBM distribution and abundance revisited

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ABSTRACT
Our estimates of the effect of climate on the worldwide distribution and relative abundance of diamondback moth have been revised. Using the known limits of the species' range, experimental observations and the known and inferred responses of the pest to temperature and moisture as the base data, we have parameterized a CLIMEX model to predict temporal abundance and spatial distributions of diamondback moth. We test our model by comparing the predicted diamondback moth distribution with its "known" distribution; for such a major pest the latter is very poorly defined indeed. We further analyze changes in relative abundance among years due to variable climatic conditions, using a series of long-term diamondback moth population data sets from China and England and associated weather data. Models such as this are crucial to our understanding of the reasons for changes in DBM abundance. They are also essential for the development of long-term pest pressure forecasts and allow us to begin to disentangle the effects of various management practices from the normal variation in abundance due to climate alone.

Keywords
Bioclimatic modelling, forecasts, prediction, population outbreaks, *Plutella xylostella*

INTRODUCTION
The diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), or DBM, is the most widely distributed major pest of crucifers. For such a major pest surprisingly little research has been conducted on its long term population dynamics and the forecasting of outbreaks (but see Zalucki and Furlong 2008). For a pest manager, being able to predict pest species abundance and distribution ('pest pressure'), and its timing and level, is crucial to both strategic planning and tactical decision-making (e.g. Maelzer *et al*. 1996). Phenological models, based on insect physiological time scales, have been relatively successful at predicting the timing of insect population peaks (e.g. Mohandass and Zalucki 2004; Collier and Finch 2004) and may be useful for strategic timing of sampling and control measures. Forecasting pest levels is more problematic because many factors influence abundance (see e.g. Yonow *et al*. 2004; Zalucki and Furlong 2005; Muthuthantri *et al*. 2010). For all major pest species, effective and timely forecasts would be useful for determining insecticide budgets, hiring additional crop scouts or making strategic decisions on which crops to plant. Long-term forecasts of abundance would be especially useful for major pest species such as DBM. Abundance can vary greatly among years. Outbreaks of the increasingly insecticide-resistant DBM can make pest management difficult; a situation which can be exacerbated if there are insufficient supplies of insecticide for distribution (Zalucki *et al*. 2009).

Zalucki & Furlong (2008) analyzed the seasonal abundance of DBM in Gatton, Australia and relatively recent outbreaks of the species in the British Isles and developed a CLIMEX model which predicted the worldwide geographic distribution of DBM. Here we refine that model and make more specific predictions of the poorly known geographic distribution for what is such a 'well-known' insect pest. We predict the species seasonal phenology for a greater number of sites across the species range and present a simple analysis of long-term abundance data for Hangzhou, China. Well-constructed models that realistically describe the effects of climate on abundance are not only useful for forecasting, but also aid in the interpretation of population changes and impacts of management practices on pest populations.

If climate is the main determinant of where a species is likely to be found, we might expect a strong influence of climate variability or weather on the temporal variation in abundance at a given site (Zalucki and Furlong 2005, 2008; Zalucki and van Klinken 2006; Lawson *et al.* 2010). CLIMEX is generally used to predict the suitability of a site for a species based on long-term average conditions and estimated responses of the species to seasonal variation in temperature and moisture. If we have the long-term daily weather data for a site (these are the data on which a site's climatic characteristics are based) we can use the estimated species response to climate variables to infer the variation in suitability (fluctuations in the various indices) at the site over time, based on the observed climate record (see also Zalucki and van Klinken 2006). Essentially we generate a model of likely seasonal population growth and mortality rates based on climate alone, using information on a species' geographic distribution and its seasonal phenology at given sites to derive the species responses to climatic conditions, or its climatic "niche".

MATERIALS AND METHODS
The rationale behind CLIMEX has been described many times and we do not repeat it in detail here (see e.g. Yonow and Sutherst 1998; Zalucki and Furlong 2008).

CLIMEX calculates a growth index (GI), analogous to population growth rate, which describes the potential of the population to increase at a location. The growth index is calculated weekly and is a product of temperature (TI) and moisture (MI) indices. For both TI and MI there is a range of conditions of temperature and moisture over which growth is maximal. Either side of the optimum range growth rate decreases while above upper thresholds and below lower thresholds growth ceases.

A measure of the relative suitability of a location for a species to persist is summarized in a single annual eco-climatic index (EI), which is scaled to 100.

$$EI=100 \sum GI/52*SI*SX$$

Where,

52 is the number of weeks in a year; SI are four stress indices, describing the species response to the extremes of cold (CS=Cold Stress), heat (HS), dry (DS) and wet (WS) conditions; SX, if needed, are interactions between extremes of these conditions; cold-dry (CDS), cold-wet (CWS), hot-dry (HDS) and hot-wet (HWS) stresses. The stress indices (SI and SX) are accumulated at a specified rate whenever conditions exceed a specified threshold.

Indices are calculated on a weekly basis for each location using standard meteorological information. A location's long term monthly average maximum and minimum temperatures, rainfall, and humidity values are used as inputs to calculate the various indices. Areas with positive values for EI are suitable for species persistence; the larger the value of EI the more suitable the location. Generally, an EI of less than 20 for a location indicates that persistence of the given species at that location is marginal. The values for parameters that describe the TI and MI components of growth, and the various stress indices (SI) and their interactions (SX) can be estimated from laboratory or field studies (Zalucki and van Klinken 2006). Unknown or poorly measured values are estimated by an iterative procedure that involves comparing the predicted distribution with the actual distribution and adjusting parameter values. Comparing the predicted and observed species distribution in an area (e.g. a continent) not used for the procedure can test the parameter tuning; see Maywald and Sutherst (1991) for details.

The CLIMEX model for DBM in Zalucki and Furlong (2008) was developed using anecdotal distribution data, the seasonal prevalence of DBM in the Cameron Highlands of Malaysia and published values for various parameters (see Table 1 for sources). Most of the temperature related parameters were taken from the study by Liu *et al*. (2002). Here we mainly adjusted the moisture related parameters (Table 1); essentially making the species more dry tolerant. This required concomitant adjustments in the dry and wet stress thresholds. Similar adjustments were made for the degree-day accumulation of cold and heat stress parameters (Table 1). We added a hot-wet interaction stress to reduce EI for tropical regions in Africa (Congo and West Africa). In the current paper we use CLIMEX version 3 (Sutherst and Maywald 2005), whereas we used version 1 in the earlier paper (Zalucki and Furlong 2008).

RESULTS AND DISCUSSION

Test of the CLIMEX model

Predicted and actual geographic distributions

The predicted worldwide distribution, mapped as EI values (Fig 1a) and GI values (Fig 1b) based on this set of parameters (Table 1) agrees quite well with the known worldwide distribution (Fig 1c). Regions with values of EI below ca 20 are probably marginal for long-term persistence of a species; essentially such conditions are associated with the edges of the species core distribution (Fig 1a). Note the increase in the species range when GI values are plotted (Fig 1b). This essentially represents the maximal range that DBM can exploit by migrating from its core range and colonizing these locations when prevailing conditions result in a positive GI. However, such migration will be highly variable from year to year (see below). Our CLIMEX prediction provides a lot more information on the distribution of DBM than the old CAB map (Fig 1c) which is likely an amalgam of EI, GI and anecdotal reports.

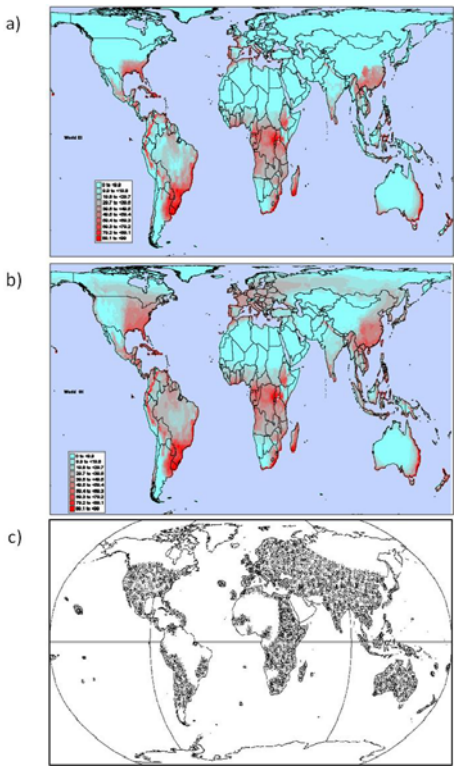

Figure 1. Predicted worldwide distribution of DBM. a) Values where GI is positive denote the core of the species range and year-round occupation b) Regions where GI is positive indicate the potential migratory seasonal range c) The 'actual" distribution redrawn from the CAB map.

Table 1. Original (Zalucki and Furlong 2008) and modified CLIMEX parameter (Param) values, their definition and sources that gave the best visual fit to the distribution of *Plutella xylostella* in Australia and Africa (see text for details).

Limiting process	Param	Original values	Changed to	Units	Definition	Source
Temperature						
	DV0	7		^{O}C	Lower temperature threshold; below this temperature the population will not grow	Liu *et al*. (2002)
	DV1	14		^{O}C	Lower optimum temperature	
	DV2	28		^{O}C	Upper optimum temperature; between DV1 & DV2 population growth is maximal	
	DV3	34	38	^{O}C	Upper temperature threshold; above this temperature populations will not grow	
Moisture						
	SM0	0.05		%	Lower soil moisture threshold; below this moisture level the population will not grow	Fitted
	SM1	1	0.5	%	Lower optimum soil moisture	
	SM2	1.5	1.25	%	Upper optimum soil moisture; between SM1 & SM2 population growth is maximal	
	SM3	2.5	1.75	%	Upper soil moisture threshold; above this moisture level populations will not grow	
Cold Stress						
	DTCS	15	12	Degree Days	Threshold number of degree-days below DV0 at which cold stress begins to accumulate.	Liu *et al*. (2002)
	DHCS	-0.0005			Rate at which cold stress accumulates when the threshold number of degree-days below DV0 (DTCS) is reached	Fitted
	TTCS	4		^{O}C	Temperature threshold at which cold stress begins to accumulate	Fitted
	THCS	-0.0001	-0.0005	/^{O}C/day	Rate at which cold stress accumulates when Tmin <TTCS	Fitted
Heat Stress						
	TTHS	30	38	^{O}C	Temperature threshold at which heat stress begins to accumulate	Liu *et al*. (2002)
	THHS	0.0005	0.001	/^{O}C/day	Rate at which heat stress accumulates when Tmax > TTHS	Fitted
	DTHS		1	Degree Days	Threshold number of degree-days above DV3 at which heat stress begins to accumulate.	Liu *et al*. (2002)
	DHHS		0.005		Rate at which heat stress accumulates when the threshold number of degree-days above DV0 (DTHS) is reached	Fitted
Dry Stress						
	SMDS	0.2	0.05	%	Soil moisture threshold at which dry stress begins to accumulate	Fitted
	HDS	-0.005		/day	Rate at which dry stress accumulates when SM is below SMDS	

Table 1 (continued)

Limiting process	Param	Original values	Changed to	Units	Definition	Source
Wet Stress						
	SMWS	2.5	1.75	%	Soil moisture threshold at which wet stress begins to accumulate	Fitted
	HWS	0.002	0.05	/day	Rate at which wet stress accumulates when SM is above SMWS	
Hot-Wet stress						
	TTHW		30	^{O}C	Temperature at which hot-wet stress begins	Fitted
	MTHW		1.25	%	Soil moisture at which hot-wet stress begins	
	PHW		0.05		Rate at which hot-wet stress accumulates once Tmax > TTHW & soil moisture > MTHW	
Limiting Conditions						
	PDD	268		Degree Days	The number of degree days above DV0 required to complete a generation	Liu *et al*. (2002)

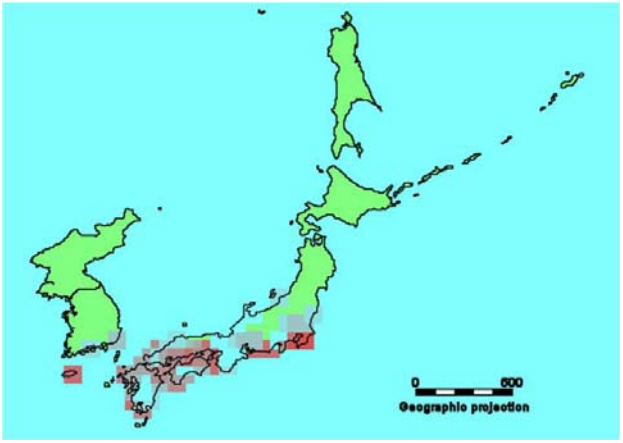

Figure 2. Predicted geographic distribution of DBM in Japan. Values where EI is positive (denoted in red and pink) represent the core of the species range and year round occupation.

The fine scale predicted distribution of DBM based on EI in Japan agrees well with observations of the species' actual distribution in this region (Fig 2). DBM does not persist in northern Japan, but does persist in southern regions (Honda 1992) from where it annually reinvades northern regions of the country. Again the geographic distribution in Japan is a prediction based on our model parameter values; it was not used to generate the model. Similarly the species does not generally persist in Korea but reinvades each year, most likely from China, and populations build up when GIs are suitable in the spring-summer (data not shown, see Furlong *et al*. 2008). Finally, the northern range limit of seasonal breeding in China (Fig 1a) appears to agree well with observations there (Fang Xia, pers. comm.)

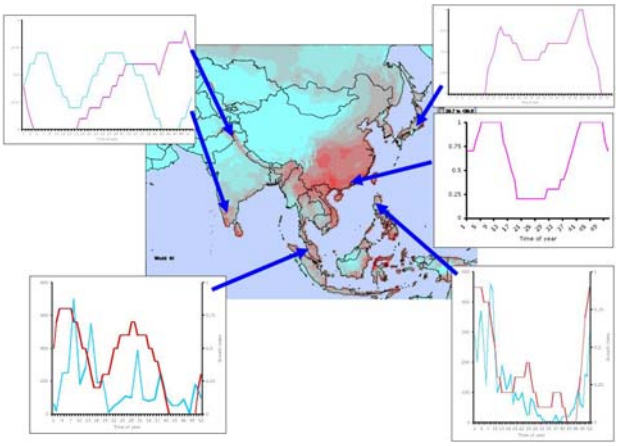

Figure 3. The geographic distribution of DBM in Asia based on EI values generated by a CLIMEX model and the seasonal phenology based on weekly GI values for a location in Japan, Philippines, southern China, Malaysia and north and south India. For Philippines and Malaysia actual data (blue lines) is also shown.

Predicted and actual seasonal phenologies

CLIMEX can be used to predict average season phenology at a site in terms of weekly growth indices. We have done so for various locations in Asia using climatic data for an average year (Fig 3); there is reasonable agreement between observed abundance and predicted growth indices. In Northern Luzon, The Philippines and Cameron Highlands, Malaysia the predicted seasonal phenologies for DBM agree well with published abundance data (Fig 3). Predicted seasonal

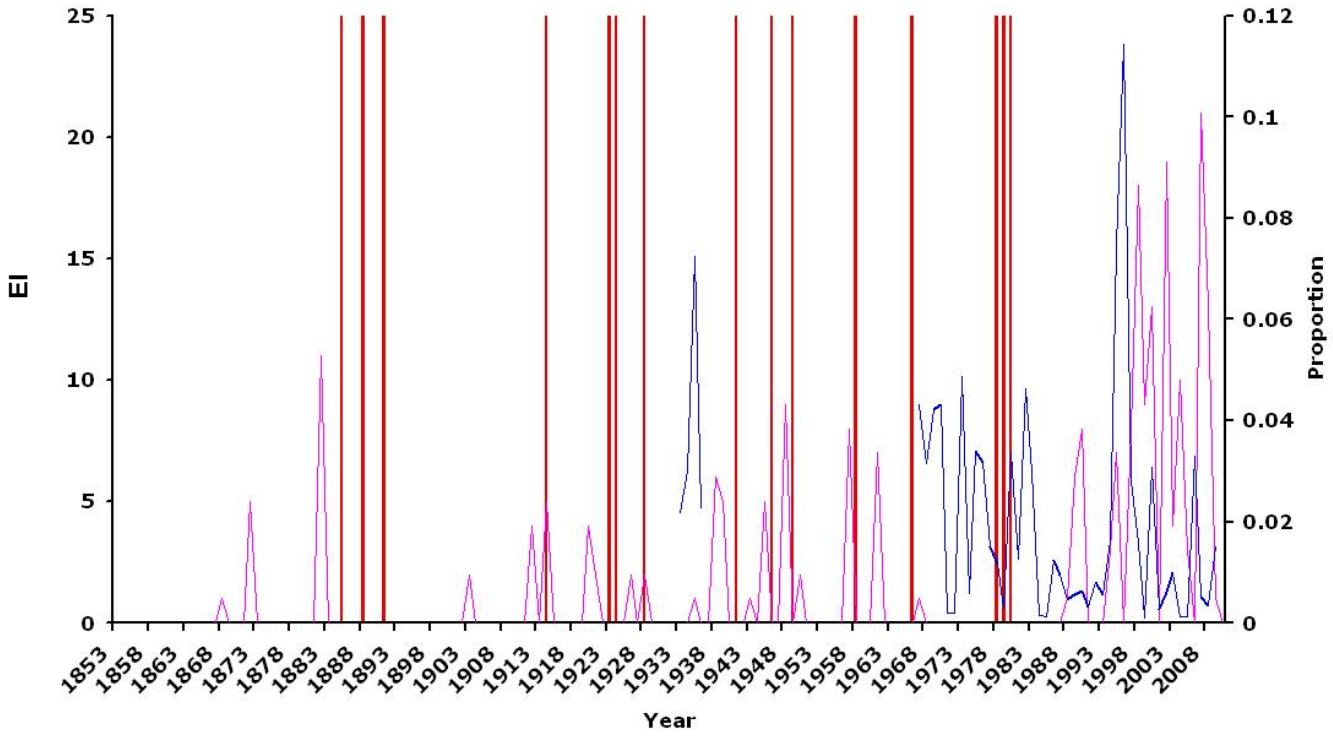

Figure 4. Annual EI values (pink lines) for Oxford, England from 1853 to date. Also shown are the years in which "outbreaks" have been recorded (red bars) as well as light trap data at Rothamsted (blue lines) expressed as proportion of total catch.

phenology in Japan, southern China and in north and south India agree with published notions of when the species is a "problem" (see previous DBM workshop proceedings). There is nothing particularly special about the species biology and ecology at specific locations throughout its range; all these predictions are based using a single set of parameters and site specific climate data.

Estimates of long term abundance

As in Zalucki and Furlong (2008) we use the CLIMEX parameter set for DBM to generate a long series of suitability estimates for locations based on climate records. We used the British Isles as a case study partly because data is available and partly because outbreaks of DBM that occur most likely migrate from elsewhere. The years in which outbreaks were recorded in the British Isles were taken from the summaries in Chu (1986). Outbreaks of DBM in the British Isles are believed to be driven by migration from elsewhere in Europe, most likely the low-countries (Chapman *et al.* 2002) and parts of southern Scandinavia and Estonia (Shaw and Hurst 1969).

Using a long series of weather data from Oxford (maximum and minimum temperatures and rainfall from 1853 to 2010) we generated yearly EI values and marked the known outbreak years, as well as light trap data for one site in recent years (Fig 4). The EI are low (generally less than 20) suggesting that the species is at the edge of its range in this region. Outbreaks are not associated with positive EI years but are most likely the result of migration, as argued elsewhere (see Zalucki and Furlong 2008). Note that EI has become more consistently positive in recent years and we can start to expect persistent populations and greater DBM pest problems in the UK. This may well represent the effects of global warming (see Sutherst *et al.* 2011).

For Hangzhou, China, we analyzed a discontinuous set of light trap data for DBM from 1976-1979, 1983, and 1986-1990. Abundance varies a great deal with very low catches in 1983 and 1986 and very high catches in 1977 and 1987 (Fig 5). We used the difference (change) in CLIMEX GI indices from one week to the next (Gi+1-Gi) as an estimate of population growth rate. Using the date of first capture of moths in the light trap record and the growth rate estimates from five weeks earlier (i-5) we generated predicted population changes for each year of record (Fig 5). There is a close fit between these predicted values and actual population data; in general our crude model "predicts" population peaks, or indicates that there could have been a peak (at least as far as climate suitability was concerned) but that it failed to materialize. Is this the effect of planting, insecticides etc at a landscape level? The exceptions are the two peak years. We wonder if the late autumn peaks do not represent migration from elsewhere, presumably northern China?

The CLIMEX approach to population modelling is very broad and crude. Insects have discrete generations with known temperature-mediated development times. Different stages have very different susceptibilities to extreme climate variables of temperature and rainfall. These stages may or may not be available in the population when the events occur. CLIMEX in a sense assumes they are available. We have consequently

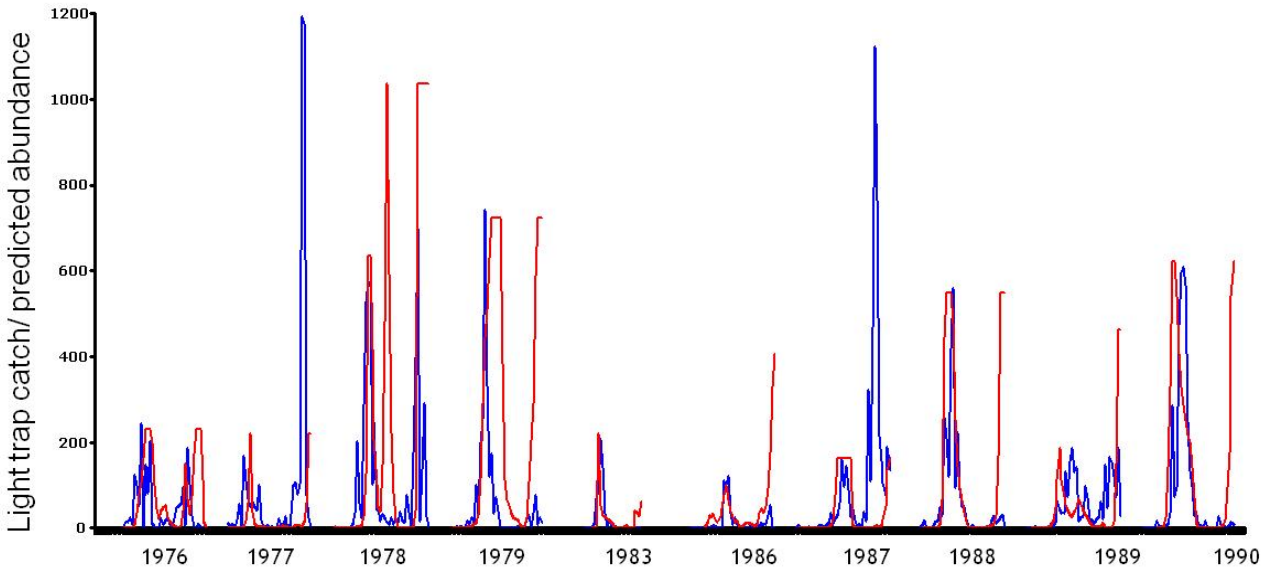

Figure 5. Observed DBM light trap catch (blue line) and predicted abundance (red line) based on a CLIMEX model for a number of years in Hangzhou, China (see text for details of the model).

started to develop and calibrate a full stage structured population model for DBM implemented in DYMEX (see Yonow *et al*. 2004; Muthuthantri *et al*. 2010 for examples of the approach).

CONCLUSIONS

Using a CLIMEX-based modelling approach, it is possible to make predictions of where a species may be found and in what relative abundance. These predictions can then be tested to shed light on the influence of climate and other factors on the species' abundance and distribution. Concordance between the predicted and observed distributions may indicate that climate influences the species' geographic range. Discrepancies either indicate that other factors need to be included (e.g. host plants), or that the model being tested is not appropriate.

CLIMEX has been used extensively in biological control programs and pest risk analysis to predict potential distributions of various species (for a brief review see Zalucki and Furlong 2005; Lawson *et al.* 2010; van Klinken *et al*. 2009). However, this method has not yet been used widely to model population changes of a species (but see Zalucki and Furlong 2005; Zalucki and van Klinken 2006; Zalucki and Furlong 2008).

The CLIMEX parameter set integrates data on species distribution into a single model. This approach may be used to generate a climate driven null model for the abundance of a species at a site over time. Such models, that enable one to infer likely species abundance based on climate, are critical to testing effectiveness of management strategies such as area wide management and the planting of transgenic crops (see e.g., Carriere *et al.* 2003; Zalucki and Furlong 2005), as well as predicting temporal dynamics and outbreaks of pests (e.g. Maelzer *et al*. 1996). Historical climate data can be used to predict both the expected changes in range and variation in the seasonal and annual temporal dynamics of the pest, and therefore of likely pest status. We have done so for a long series of data at Oxford and Hangzhou. For the former location migration appears to be important but this can be very difficult to predict. For the latter location, climate may well generate a great deal of variation from year to year, but migration may be important both at the beginning and end of the season.

It is possible to derive a model for the population dynamics of a species driven by climate and other factors (e.g. predation, soil type, host plant availability) using other modelling tools (e.g. DYMEX, Maywald *et al*. 1999). Such models may simply attempt to predict seasonal phenology based on the developmental biology of the insect (e.g. Mohandass and Zalucki 2004) or include a comprehensive description of what is known to impact on a species' population dynamics (e.g. Yonow *et al*. 2004). We have developed a simple DYMEX model for DBM and begun an analysis of long-term abundance data in South Africa. Of course abundance of plant pests or phytophagous insects will not only be influenced by the weather, but also by the availability and quality of the host plant and their own natural enemies (e.g. generalist predators etc). Only experimental manipulation of populations can reveal the relative contribution of such factors to reproduction and mortality (e.g. Furlong *et al*. 2004ab; 2008).

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Rising to the challenge: a national project on sustainable control of DBM in China

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ABSTRACT
Over-wintering and migration, insecticide resistance monitoring, resistance mechanism, resistance management and sustainable control of diamondback moth were carried out in South-, Southeast-, North-, Southwest- and Middle of People's Republic of China. The results indicated that the number of generations of diamondback was increasing from north to south. The population peak is late from south to north. There are one to two peaks each year. The population is affected by temperature, rainfall, natural enemies, etc. The results of resistance monitoring and management studies showed that the resistance to 11 selected insecticides is high. There were huge differences among different areas. The resistance was high in South, Southwest and Southeast and on rise in Middle and North. The resistance management strategies were built according to the monitoring. The result of over-winter and migration studies illustrated that it was a borderline of over winter from Wuhan to Zhu Madian. The diamondback moth could migrate following two patterns. One was "move in and move out", and another was "move in and settle down". The results of resistance mechanism clarified that it was different style of inheritance to Bt, abamectin, chlorfenapyr, indoxacarb and diafenthiuron. The methods and strategies of diamondback moth integrated management were for each region. Forty-five base demonstrations were established for the technologies to be demonstrated for managing diamondback moth in South, North, Middle, Southeast and Southwest of PR China. During these demonstrations, more than 160,000 technical handbooks were delivered, and 51,900 farmers producing brassicas in 24,000 ha farms and orchards were trained, and obvious economic, ecological and social benefits were obtained subsequently.

Keywords
diamondback moth, resistance detection, resistance mechanism, resistance management, sustainable control

INTRODUCTION
The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), infesting over 40 plant species, is a well-known and destructive insect pest of brassica crops worldwide. In China, DBM is now the most serious threat to vegetable brassica production in South China, and canola production in West China, although its occurrence has been found in many other provinces, as well. The DBM larva can damage the cruciferous plants by feeding and mining during the whole growing season. Outbreaks of DBM in China sometimes cause more than 90% crop loss (Chin et al.1990). In Shanghai (PR China), when no insecticides

were applied to control DBM in 1992 and 1994, the losses of summer cabbage were 99% and 80%, respectively compared with the insecticide-treated plots (Zhao et al. 1996).

In 1953, DBM was reported by Ankersmith as the first crop pest in the world to develop resistance to DDT in Java, Indonesia (Ankersmith 1953). The resistance development to several insecticides had been reported in the Philipines (Barroga 1974), Australia (Altman 1988), Hawaii (Tabashnik et al. 1987), Malaysia (Syed, 1992), Japan (Hama, 1992), North America (Shelton et al. 1993), Thailand (Kuwahara et al. 1995). In 1992, Cheng and Tang reported the resistance of DBM to insecticides in PR China. Up to now, because of the extensive selection pressure and its high potential for developing resistance, DBM had developed resistance to the major types of insecticides, including organophosphates, organochlorines, carbamates, synthetic pyrethroids, some insect growth regulators, and microbial insecticides such as *Bacillus thuringiensis* (Liu et al. 1981; Miyata et al. 1992; Tabashnik et al. 1990; Talekar and Shelton 1993). In PR China, chemical control is still the main way to manage the DBM. The massive abuse of chemical insecticides was leading to the rapid development of insecticide resistance. Due to insecticide resistance and eventual control failure of DBM, economical production of crucifers had become almost impossible in certain Chinese areas (Liang et al. 2001; Liu et al. 2005).

In the past two decades, Chinese entomologists had carried out more research on DBM in PR China, including occurrence, resistance, molecular genetic mechanism and IPM, etc. Some measures had been developed in cultural control, chemical control, physical control and biological control, although systematic demonstration was lacking. In order to produce better quality brassica vegetables and canola, it is necessary to build and demonstrate new integrated technologies according to the local conditions of different areas. In 2008, a national project, *the Special Fund for Agro-scientific Research in the Public Interest*, supported the research on sustainable management of DBM. The project is a group effort involving 117 entomologists from ten institutes or universities, including nearly all DBM experts in PR China. The group covers five geographic areas, *viz*., south, south-west, east, north and center. The significant progress made in the project during the past three years is presented here.

Investigation on population dynamics in five geographic regions

Population dynamics of DBM in 14 locations from 10 provinces in south, south-west, east, north and center China were investigated. The results showed that there were more than 20 generations in south China and it was only 2-3 generations in north-east China. There were one or two population peaks in different areas during spring or autumn. The time of initial peak occurred during February – March in South China and May – June in North-East China. The spring peak was higher than the autumn one in North China, whereas the two peaks were similar in South China.

In 2010, the Chinese DBM population was significantly low, and it could be due to the changes in climate, natural enemy population and cropping systems. In terms of the climate, mean spring low temperature and summer high temperature was one of the reasons. At the same time, the population was also controlled by a tremendous amount of rainfall. In addition, the natural enemies including *Diadegma semiclausum*, *Cantheonidea furcellata* and *Erigonidium graminicola*) and the cropping systems including rotating rice, corn and shallot in summer were effective in managing the DBM.

Current status of insecticide resistance in DBM in PR China

DBM field populations were monitored for their resistance to 11 selected pesticides (Table 1) in 34 locations in south, south-west, east, north and center China following a standard method. The results showed that the resistance was serious in all locations. There were significant differences in resistance to an insecticide among the study sites. The resistance was more serious in south, south-west and east China than other parts. The resistance in Danzhou (Hainan Province) was the most serious. High resistance is correlated to the population differences and over-use of insecticides. It is very difficult to plant vegetable brassicas in south China during summer because of high resistance in DBM. So, the farmers in the north or west China prefer planting vegetable brassicas during summer. However, it is a remarkable fact that the insecticide resistance in DBM in different geographical regions is gradually decreasing year by year from 2008 to 2010, which proves that the IPM technologies demonstrated and promoted through the project are effective.

Over-wintering and migration

Some research work was also carried out on the nature and north limit of over-wintering. The super-cooling point was high with the growth of larva, and the mean was -13.72°C. The super-cooling point was lowest for the pupa. The freezing point had the same trend as the super-cooling point. The research on the north limit of hibernation in PR China showed that the north limit was the area from Wuhan to Zhu-ma-dian based on the investigation on over-wintering in 19 locations.

The genetic differences among 10 geographic DBM populations were lower. Hence, DBM could migrate from south to north. The adult migration and climate condition were monitored in 19 locations. The results showed that there were two models of migration, *viz*., *move in and move out* (*e.g.*, in Wuhan and Nanjing) and *move in and settle down* (*e.g.*, Datong and Shenyang). Simultaneously, the resistance of DBM in different locations was analyzed and found that it was very similar to different insecticides among Wuxue (eastern Hubei province), Luoyang (western Henan province) and Beijing (northern China), which could be due to migration.

Table 1. List of insecticides for which the DBM resistance was monitored

English Common Name	Active element
Alpha-cypermethrin	5.16%
Spinosad	2.50%
Fipronil	5.46%
Indoxacarb	15.00%
Abamection	2.00%
Chlorfluazuron	5.59%
Diafenthiuron	21.84%
Tebufenozide	10.64%
Chlorfenapyr	10.26%
Cartap	97.10%
Cry1Ac	3.00%

Inheritance and mechanism of resistance to insecticides

Identifying the resistance mechanism to five insecticides, *viz.*, Bt-toxin, abamectin, fipronil, indoxacarb and diafenthiuron are in progress. A rapid resistance screening method for Bt-toxin and abamectin will be built in the near future. The resistance mechanism to three insecticides has been briefed below.

Inheritance and mechanism of resistance to Bacillus thuringiensis

The inheritance to Cry1Ac was incomplete recessive inheritance on single-gene. There was cross-resistance between Cry1Ac with Cry1Aa, Cry1Ab and Cry1F; and no cross-resistance with Cry2Ab, Cry1B and Cry1C. The main mechanism of resistance was the incompatibility of Bt-toxin with receptors. Using the AFLP markers, an early detection system for Bt resistance is being built.

Inheritance and mechanism of resistance to abamectin

The inheritance to abamectin was incomplete dominant inheritance on multi-gene. There was a high level cross-resistance between abamectin and emamectin benzoate, low level cross-resistance with spinosad and fipronil, no cross-resistance with indoxacarb, metaflumizone, chlorfenapyr, Cry1Ac, chlorfluazuron, tebufenozide and beta-cypermethrin. The main mechanism of resistance was over-expression of GluCl receptor alpha gene.

Inheritance and mechanism of resistance to fipronil

The inheritance to abamectin was incomplete dominant inheritance on single-gene. There was no cross-resistance between fipronil and abamectin, monosultap, indoxacarb, spinosad, metaflumizone, chlorfenapyr, Cry1Ac, chlorfluazuron, tebufenozide, chlorpyrifos and beta-cypermethrin. The main mechanism of resistance was gene mutation of A302S in PxRdl gene.

SOME KEY TECHNOLOGIES FOR MANAGING DBM IN PR CHINA

Cultural control

While comparing three different cropping systems *viz.*, continuous cropping, rotation with either rice or fallow for managing DBM, the result showed that the population was highest in the continuous cropping field despite spraying insecticides. The two other rotations significantly reduced the DBM population because of breaking the availability of host-plants.

Biological control

We intend to assess the safety of different insecticides to three natural enemies *viz.*,, *Diadegma semiclausum*, *Trichogramma* sp. and *Erigonidium graminicola*. As a first step, mass production technique for *Diadegma semiclausum* had been developed. A method to mass-culture *Cantheonidea furcellata* on the larva of tobacco cutworm is being developed. In addition, the group collected 143 species of entomopathogenic fungi and tested 11 of them for controlling DBM.

Mass trapping devices

The group created a new insect-ware to attract the adults. Using the ware, six sex pheromone lures from different companies were tested in three geographic areas, and found that different geographic DBM strain reacted differently for the same pheromone. As an important breakthrough, a high voltage machine for killing moths was developed to be used in the field, and it was effective to kill adults in the field.

Insecticide rotation strategies

It was very important to use chemical pesticides properly and delay resistance development. According to the results of resistance monitoring in different locations, insecticide rotation strategies were developed. These were made available in the form of a handbook. The handbook could guide farmers to use insecticide effectively. The following was an example of insecticide rotation strategy in Guangdong province.

Table 2. The insecticide rotation strategy in Guangdong province

Period	Rotation Strategies
January - April	Spinosad, Cartap, Diafenthiuron, Metaflumizone
May - September	Fallow or rotating rice, corn, shallot, bean etc.
October - December	Indoxacarb, Chlorfenapyr, Metaflumizone, Chlorantraniliprole

SUMMARY AND FUTURE PROSPECTS

The methods and strategies for integrated management of diamondback moth were developed for each region. Forty-five base demonstrations were established for the technology demonstration in South, North, Center, Southeast and Southwest of PR China. During these demonstrations, more than 160,000 technical handbooks were delivered, and 51,900 farmers producing brassicas in 24,000 ha farms and orchards were trained, and obvious economic, ecological and social benefits were obtained subsequently. The group will pay more attention to control the DBM on canola crops in the next phase. There is still a long way to control DBM in the future.

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Progress and challenges in the Bt brassica CIMBAA public/private partnership

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ABSTRACT
The public/private partnership, *Collaboration on Insect Management for Brassicas in Asia and Africa (CIMBAA)*, was formalized in 2005 with the intention of holding and disseminating the eventual transgenic Bt plant products, capable of controlling diamondback moth, from public sector hands. The efficacy of the Cry1B/Cry1C Bt protein combination for a range of brassica pest species was demonstrated. Cabbage and cauliflower transformations were completed and the plants imported into India for selection and testing in 2006. Agronomy, protein expression, molecular biology and insect control efficacy trials were undertaken from 2006 in north India (Murthal near New Delhi) and in south India (Bengaluru). This resulted in the selection and confirmation, by May 2009, of one preferred cabbage line and one preferred cauliflower line (Elite Events) and a range of hybrids derived from them. Control efficacy for the whole suite of pest Lepidoptera is excellent (see Kaliaperumal *et al*. this volume). A published survey of consumers in four Indian cities suggests good acceptance of Bt vegetables at the right price and published farmer surveys confirmed the need for and acceptability of Bt brassicas for economic and environmental reasons. Preliminary work on the formal biosafety studies has been completed. However, AVRDC - The World Vegetable Center withdrew from CIMBAA in spring 2009 citing inexperience in the stewardship and licensing of GM crops and concerns on the uncertain and potentially open-ended liabilities. The three University partners (Cornell University, University of Melbourne and University of Greenwich) concluded that they could not take this role and Cornell University also withdrew from the partnership. A search for other acceptable public sector partners willing and able to hold the IP and undertake the licensing / stewardship role was finally unsuccessful. A review of the possible ways forward was undertaken in early 2010 without identifying clear ways out of the impasse and the CIMBAA Steering Committee decided to cease the development of the CIMBAA plant lines in April. Field work ceased immediately, followed by the ending of laboratory work with the plants in Sept 2010. Increasingly stringent industry stewardship standards which make the intended sharing of the material difficult; the ill-defined and potentially open-ended nature of 'liability' especially in relation to the likely movement of seeds to jurisdictions beyond India and the uncertain timelines and costs of the Indian regulatory process following the delay in late 2009 in releasing Bt eggplant which had satisfied all the regulatory requirements, have all combined to halt the development of a technically and environmentally excellent product at a very advanced stage. Discussions are underway with the Indian Council of Agricultural Research as to how the benefits of this work may yet be captured for the benefit of the poorest farmers and consumers.

Keywords
Bt brassicas, diamondback moth, CIMBAA, stewardship, liability

INTRODUCTION
The Collaboration on Insect Management for Brassicas in Asia and Africa (CIMBAA) was formally set up in 2005 following three years of preliminary studies on the potential for transgenic Bt control of diamondback moth and other caterpillar pests of brassicas in the developing world. The aim was to find an 'in the seed' solution to the intractable problems of diamondback moth in vegetable brassicas.

The public sector partners were to:

- undertake the bioefficacy studies
- the crop based safety studies (ecotoxicity, impact assessment, outcrossing studies)
- undertake the molecular characterisation of the plants
- bacterially produce and purify the proteins
- undertake socio-economic studies
- be responsible for the communication of the project to stakeholders.
- be the holder of the regulatory dossier
- undertake the licensing of the plants
- consequently be responsible for liability

The private sector partner was to:

- undertake the transformation activities
- undertake contained and confined field testing

- undertake breeding and trial activities
- undertake protein based safety studies
- obtain the regulatory trial permits

CIMBAA reported on the rationale for the development and deployment of stacked gene Cry1B/Cry1C, and the initial import of transformed cabbage and cauliflower lines into India for testing and selection, in the proceedings of the 5th International Workshop in this series in 2006 (Russell et al. 2008).

Phase I of the partnership (2002-04) dealt with country needs assessments, technical feasibility studies, gene selection and stakeholder agreement.

Phase II (2005-2006) formally established the partnership, demonstrated the need for the product, developed the transgenic plant material, undertook proof of concept lab and field testing and undertook preliminary studies on biosafety.

Phase III (2007-10) focused on the efficacy of the Bt plants against a range of key pests, selection of plants for commercialization and developing an IPM context for the material.

Phase IV (2011-) would be the undertaking of formal regulatory studies in India, followed by submission of the regulatory dossier for release approval in India and for biosafety/import approval in other major global regulatory regions.

This paper describes progress in phases II and III and discusses the situation *vis a vis* Phase IV.

The CIMBAA public sector collaboration partners were the University of Melbourne (Australia), Cornell University (Entomology Department and Office of International Programmes) (USA), the Natural Resources Institute of the University of Greenwich (UK) and AVRDC- The World Vegetable Center (Taiwan), with Nunhems Pvt. (Netherlands and India), the vegetable seeds division of Bayer Crop Science, as the private sector seed company developing the transformed plants.

The 2008 paper summarized the progress of the work to 2006 with the Bt proteins Cry1B and Cry1C selected in 2003 on the basis of earlier research by Zhao et al. (2001) and Mohan and Gujar (2002), which suggested good efficacy and critically no cross resistance between these two proteins. Zhao et al (2003) showed that stacked Bt proteins were more effective in delaying resistance compared to single proteins in a rotation or mosaic. Building on and expanding earlier work reported in Russell et al. (2008), Grzywacz et al. (2010) reviewed current control methods for brassica pests in Asia and Africa and concluded that effective Bt transgenics could play a very useful role and should be complimentary to other IPM practices including the conservation of important parasitoids such as *Diadegma semiclausum* and *Cotesia plutellae*.

CIMBAA STRUCTURE

The CIMBAA Steering Committee comprises the directors of the collaboration's partner institutions and has the oversight and decision making responsibility, meeting twice a year or as required. The project management team with day to day responsibility for the work comprises leading scientists from each of the partners, with coordinators from the public and private sectors who also act as secretaries to the Steering Committee. The project management team discusses monthly by conference call. CIMBAA also set up advisory panels to both help guide the on-going work of the project and to ensure that the work was to the highest international standards. The International Advisory Panel comprises past and present Directors General of Consultative Group on International Agricultural Research (CGIAR) or other international agricultural research centres, institutes (ICIPE, IRRI, CIMMYT), and of the Indian Council for Agricultural Research (ICAR), the immediate past Director of Research of Nunhems, plus a specialist in gene flow science. The International Advisory Panel has provided advice and comment to the Steering Committee on a number of technical and process issues.

The Indian Advisory panel has served two functions. It has provided detailed technical guidance on the studies to be undertaken by CIMBAA and has provided a mechanism for the project to ensure that the work is understood and approved of by senior Indian government scientists. The panel has been chaired by the Executive Director of The Energy and Resources Institute (TERI), who has had a leading role in the communication aspects of CIMBAA. The committee's makeup has varied over the years but has included an Assistant Director General (Crop Science) from ICAR, Directors of the Department of Biotechnology (DBT), National Research Centre for Plant Biotechnology (NRCPB), Indian Agricultural Research Institute (Divisions of Entomology, Vegetable Science and Horticulture), National Bureau for Plant Genetic Resources (NBPGR) and the National Bureau of Agriculturally Important Insects amongst others.

ACCEPTANCE OF NEED FOR AN "IN THE SEED"SOLUTION

Farmer pest control studies

A number of studies for CIMBAA, summarized in Grzywacz et al. (2010), demonstrated the potential value to farmers in Africa and Asia of Bt brassicas (see also Badnes-Perez and Shelton 2006). Specifically for India, a study undertaken in connection with CIMBAA (Weinberger and Srinivasan 2009), confirmed the unsatisfactory state of current insect control on cabbage and cauliflower. 97% of the 300 farmers interviewed in Gujarat, Karnataka and West Bengal regarded insects as their number one production problem and all of these farmers used chemical pesticides (72% used nothing but pesticides). Thirty seven different pesticides were in use and 66% of farmers were routinely spraying insecticide

mixtures. One third of farmers were spraying even if there was no insect damage on the crop and only 8% considered pest numbers before spraying. Given that farmers were routinely spraying every 3-4 days and often more frequently, especially on cauliflower, it is not surprising that 75% had health issues related to pesticide use in the preceding year and indeed 2% of farmers had been hospitalized. The average cost of pest control in cabbage was US$ 141/ha and US$ 316/ha for cauliflower (10-30% of total production costs).

Stakeholder Support

A key workshop held in New Delhi in 2005 entitled *Public-private partnership in the use of Agribiotech for sustainable solutions to brassica pest problems* established the scientific commitment to genetic solutions. Dr. Trivedi of the National Centre for IPM said that seed would be the most important component of IPM, and Dr G. Kalloo, Deputy Director General of ICAR confirmed that gene pyramiding is a major drive of ICAR (Srinivasan et al. 2005). The potential role of public/private partnerships in Agricultural biotechnology was strongly endorsed by a panel including the Indian Prime Minister, Dr. Manmohan Singh, the leader of the Indian Green Revolution Prof. M.S. Swaminathan and the Minister of Science, Sri Kapil Sibal, at the International Conference on Agriculture for Food, Nutritional security and Rural Growth in New Delhi in May 2006.

Consumer study

Given the global concerns over GM food crops, a study of 645 households in two large urban centres (New Delhi and Bengaluru) and two regional centres (Kolar and Barddhaman) was undertaken in 2006-07 (Krishna and Qaim 2008). It found that 80% of consumers recognised pesticide residues on vegetables as a significant problem. The concept of Bt vegetables was new to most interviewees, but, following a standard explanation, 68% supported the introduction of Bt vegetables if they cut residues (and provided they were approved as safe by the Indian regulatory authorities). Most of the 17% of households who strongly opposed to GM vegetables would buy them if they were significantly cheaper than non-GM alternatives. However, the more educated the respondent, the more likely they were to both be aware of residues and to be anti-GM foods. It seems likely that in recent years this attitude has spread further with the anti GM political agitation by some NGOs in India.

TRANSFORMATION, IMPORTATION AND PRELIMINARY TESTING IN INDIA

Some 600 primary transformants for each of cabbage and cauliflower were produced at Nunhems facilities in the Netherlands. The transformation was *Agrobacterium* mediated into cabbage and cauliflower hypocotyls and involved the insertion of cassette containing tightly linked *cry1B* and *cry1C* genes driven by subterranean clover stunt virus promoters with the *bar* herbicide tolerant gene and its PAT protein as a selection marker. As required under Indian regulations, Nunhems set up an Institutional Biosafety Committee in 2005 and applied a license for the import of Bt transgenic lines which was received in April 2006 with 85 lines released from quarantine in August that year (other importations were made later).

An initial polyhouse (plastic greenhouse) trial was undertaken on the Nunhems field site at Bilaspur near Delhi during autumn 2006 (Fig. 1). Laboratory reared diamondback moth were released onto the Bt plants and the plant performance, monitored by the India regulators, was regarded as a sufficient proof of concept to justify larger scale trials in the following seasons.

Figure 1. Government inspection of the first CIMBAA trial, Bilaspur, near Delhi 2006

EFFICACY, SAFETY, SUSTAINABILITY AND REGISTERABILITY TESTING

Expression of Cry proteins in plants

Cry protein expression levels in different tissues of the CIMBAA plants, over the growing season and in different parts of India were studied by N. Kaushik (paper in prep.) of The Energy and Resources Institute, new Delhi. In summary:

- Protein was expressed in all plant parts including cauliflower curds.
- Protein expression was fairly constant in different parts of leaves and different leaf positions on the plant.
- Expression was constant across plants from a given event but varied somewhat with the date of sampling.
- Expression in hybrids (single copy of each Bt gene) was 20-40% lower than in the Bt parents.

Susceptibility of key pests to the Bt proteins

The key lepidopteran pests of brassicas in Asia and Africa are (in approximate order of importance):

- Diamondback moth (*Plutella xylostella)*
- Cabbage cluster caterpillar *(Crocidolomia binotalis)*
- Cabbage webworm *(Hellula undalis)*
- Cabbage white butterfly (*Pieris brassicae*/ *P. rapae)*
- Cabbage leaf worm *(Spodoptera litura)*
- Cotton bollworm *(Helicoverpa armigera)*

Cutworms (*Agrotis* spp.) and the semi-looper *(Trichoplusia ni)* can be occasional problems. The key non-lepidopteran pests are aphids, especially *Brevicoryne brassicae*, *Lipaphis erysimi* and *Myzus persicae*, the mustard sawfly (*Athalia proxima leguna*) and the painted bug (*Bagrada cruciferum*).

Bioassays with pure Cry1B and Cry1C proteins in India, USA, Australia, Indonesia, Taiwan and China (Shelton et al. 2009) using leaf dip assays showed the LC_{50} values for both Cry1B and Cry1C against diamondback moth to be <1.3 ppm in all field populations tested. *C. binotalis* had LC_{50}s of >1.07 ppm for Cry1B and <1.89 ppm for Cry1C. *H. undalis* had LC_{50}s of >2.09 ppm for Cry1B and <1.37 ppm for Cry1C. *P. rapae* and *P. brassicae* were very susceptible to Cry1B with LC_{50} vales <0.15 ppm, but were less susceptible to Cry1C (*P. rapae* LC_{50} 2.68 ppm and *P. brassicae* 19.22 ppm). The prospects for control of these species with transgenic Bt brassicas was therefore good. However, *S. litura*, an increasing pest of brassicas in Asia showed less susceptibility with LC_{50}s >10 ppm to both proteins. The performance against *H. armigera*, which is a sporadic pest, was even poorer.

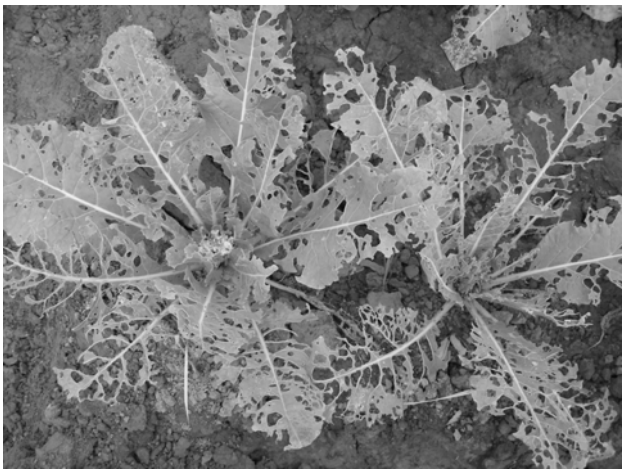
Insect efficacy trials with Bt plants

Figure 2: Insect efficacy and Elite Event selection screenhouse trial at Murthal, near Delhi Oct-Dec 2006

Fuller details of the testing of transformed plant lines and hybrids derived from them are given in Kaliaperumal et al. (this volume). Only a brief outline is provided here. Large scale screenhouse trials were undertaken in 2008 and 2009 seasons in two main production areas, on the Nunhems farms at Murthal near Delhi (Rabi season) (Fig.2) and near Bengaluru (Kharif season).

Selection of the events was made progressively using molecular and agronomic characteristics in addition to the level of control of the key insect pests. Only results from the best lines (on molecular, agronomic and insect control criteria) and their hybrids are given in Table 1. Insects were placed as eggs or neonates on homozygous Bt cabbage and cauliflower plants and on cytoplasmic male sterile (CMS) hybrids generated using plants from the best performing Bt events as one parent (and so heterozygous for Bt). Heterozygotes had typically 20-40% lower Bt expression than homozygotes. Insects were counted 48 h after placement on the plants and plant damage was scored on a scale of 0 to 4, where 0 was completely undamaged (Figure 3a) and 4 meant the plant was destroyed for any useful purpose (Figure 3b). The damage in the non-Bt control plots varied from 3.3 to 4.0 depending on species and trial. The highest survival and damage was observed in earlier trials implying that there was still some segregation in the lines.

a) Cry1B/1C transformed plant (damage score 0)



b) Untransformed plants (damage score 4)

Figure 3. Cauliflower plants onto which 40 neonate Spodoptera litura/plant were released (on white stapled card).

Table 1. Mortality of lepidopteran pests on CIMBAA Elite Event plants and hybrids in screenhouse trials in 2008 and 2009 near Delhi and Bengaluru

Rank*	Lepidopteran Pest Species	Mortality (%) *(mean damage score)*
1	*Plutella xylostella*	100 *(0)*
2	*Crocidolomia binotalis*	100 *(0)*
3	*Hellula undalis*	100 *(0)*
4	*Pieris brassicae*	A little survival on cabbage only‡ (*0.7 cabbage; 0 on cauliflower*)
5	*Spodoptera litura*	90-98% on cabbage and up to 79% on cauliflower‡ (*0.4 on cabbage and 0.6 on cauliflower)*
6	*Helicoverpa armigera*	Not well controlled but larval weights reduced >50% and little pupation (*3.3 on cabbage and 4.0 on cauliflower*)
7	*Trichoplusia ni*	100 (0)

*Rank as a pest in India

‡ No survival to pupation

Sustainability

Diamondback moth has a long history of the evolution of resistance to sprayed insecticides. In Hawaii, DBM developed resistance to sprayed Bt formulations (Mau and Gusukuma-Minuto 2004). For this reason, CIMBAA chose in 2003 to stack two Bt genes (Cry1B and Cry1C) which are both effective against diamondback moth and were thought to have different binding sites in the insect gut and so not to be cross-resisted. G. Behere of University of Melbourne and G.T. Gujar of IARI have been studying this issue under an Australia-India Strategic Research Fund grant (2008-11). The work is just completing now and is unpublished but in outline despite the 60 fold range of LC_{50}s to the Cry proteins found in 18 Indian field populations, none were much less susceptible to either Bt protein than the standard international susceptible strains. Screening of Indian strains for resistance alleles at 1 ppm (using the F_2 method) found 0.5% survival for Cry1B and no survival for Cry1C and no resistant families were able to be generated. Attempts to select resistance in DBM in the laboratory over 25 generations produced only very modest resistance to Cry1B (9 fold) and a maximum of 4 fold resistant to Cry1C. Selecting with the two toxins together was no more successful than with Cry1C alone. All resistances were highly unstable and declined rapidly in the absence of selection (*i.e.*, had a strong fitness cost). A few insects from these less susceptible laboratory strains were able to survive for a few extra days on the Bt plants but growth was severely affected and no DBM survived through pupation. The 9-fold resistant Cry1B strain showed no cross resistance to Cry1C.

Field studies by TERI in northern and southern India suggest that the alternate crop refugia areas for diamondback moth during the cabbage and cauliflower growing seasons are likely to be sufficient to contribute strongly to slowing the development of resistance by generating susceptible insects with which rare resistant insects emerging from the Bt field would be likely to mate. As at least Cry1B resistance appears to be recessive, it would appear that the 'high-dose-refugia' requirements of resistance management in the field as used for other pest/Bt crop combinations are likely to be met by DBM for Cry1B/1C.

Partial and full exclusion cage studies by TERI in Bt sprayed fields demonstrated both for DBM (V. Sharma) and *S. litura* (R. Kaliaperumal), that natural enemy numbers were high enough in Bt fields to have a major impact on survivorship of any rare surviving insects in a transgenic Bt field.

IPM context

Control of pests not susceptible to Bt toxins is still required. Ideally this should be without the foliar application of broad spectrum insecticides both for human health reasons and because it undermines the role of beneficial which are enhanced by the use of plant expressed Bts in the absence of insecticide spraying. CIMBAA has therefore been investigating appropriate IPM strategy contexts for the deployment of the Bt plants.

Aphids are the clear major pests after caterpillars, especially *B. brassicae, L. erysimi* and *M. persicae*. The timing of attack varies across India and between seasons and can be sporadic. Work by R. Kaliaperumal showed that early season (up to 45days from transplanting), imidacloprid is effective as a pre-transplant seedling drench. However pelleting imidacloprid (as Gaucho 600 FS) onto the seed avoids the operator exposure and complications arising from seedling drenches. Later in the season, a maximum of two spray applications of *Verticillium lecanii* (at 45 and 55 days post transplanting) controls any remaining aphids up to harvest.

Cabbage leaf worm (*S. litura*) appears to be strongly, but not ideally, controlled by the Bt toxins and the cotton bollworm (*H. armigera*) is rather poorly controlled. Experiments showed that both species can be well controlled (>90% reduction in larval activity) by the Bt plants in combination with the insects' respective nucleopolyhedroviruses (NPVs) at 1.04×10^9 PIB. These NPVs are commercially available. Further work to tailor packages to local conditions is required.

Characterization of CIMBAA plants for registration

Registration authorities may call for a range of appropriate studies but these normally include the following molecular and biosafety criteria.

- Single genetic locus

- No vector backbone present
- Does not disrupt neighboring genes
- The full structure and sequence of the transgenic insertion and genomic region
- Measurement of the transgenic proteins over life of the plant and in all tissues
- The stability of the transgenic and neighboring genes in all tissues
- The development of a method capable of detecting adventitious presence of 1 Bt seed in 30,000 in a wild-type seed lot
- The transgenic insertion does not create (directly or indirectly) proteins which could be allergic
- That the plant proteins are identical to those produced by the bacterial expression system used for the safety studies (protein equivalence).
- Animal toxicity studies (probably rat, chicken, goat and cow in this case)
- Target and non-target organism mortality – 'ecotoxicity'
- Simulated human digestion and exposure studies (mouse).

Work by A. Williams at the University of Melbourne has:

- Completed the pre-screening as above
- Established a method for unambiguously detecting each Elite Event
- Sequenced the Elite Event and back-up events for cabbage and cauliflower
- Undertook a preliminary sequence survey of each Elite Event and it border regions and produced a comprehensive regulatory-quality dossier report.
- Undertook the bioinformatics and allergenicity assessment on each Elite Event

No allergenicity or other molecular issues which would prevent registration of the CIMBAA Elite Events havebeen found. Simulated human gut digestion studies have been satisfactory and preliminary acute studies on mice have likewise not raised any animal or human health concerns. A full set of protocols and costs for the toxicology and livestock performance studies to be undertaken in government approved Indian institutions, was drawn up and costed by Prof. Andrew Cockburn of the University of Newcastle for CIMBAA in April 2009, with studies intended to begin from early 2010.

Preliminary studies by Prof. Anthony M. Shelton at Cornell University were able to demonstrate that there were no direct impacts of the Cry1C Bt protein on the DBM larval parasitoid *Diadegma insulare* nor on the pupal parasitoid of *Pieris spp.*, *Pteromalus puparium.* Dr. J. Romeis of Agroscope Rekenholz-Tanikon Research Station, Zurich has shown that there were no adverse effects on a representative (*Chrysoperla carnea*) of the major predatory beneficial group – the lacewings (Neuroptera) of either Cry1B or Cry1C when fed directly (see Shelton et al. 2008 for a summary). Unpublished work by Dr. Gujar of IARI found little effect on a range of life table parameters (only slightly reduced fecundity) in the predatory ladybird beetle, *Coccinella septempunctata* when fed on aphids which had been dipped in Cry1B or Cry1C protein. Field breakdown rate and soil fauna impact studies are pending.

Elite Event Selection

The performance of the various CIMBAA events and on that of the CMS hybrids (various genetic backgrounds), which were produced from them was assessed to select the Elite Events (events proposed for further breeding and eventual commercialization), plus 2-3 'back-up' lines which were retained in case any later problem came to light with the elite events.

Nunhems breeders assessed agronomic performance on 15 characters, including firmness, head size, color, openness, non-splitting, field standing capacity, etc. Molecular screening was carried out on the basis of gene copy number, segregation, border sequences and insets plus Southern and PCR tests for presence. Protein expression was assessed from the detailed ELISA data from thousands of individual plants over two seasons by Amar Diagnostics Ltd under contract to Nunhems. The pest control performance of the lines was, of course, a major determinant.

The four years of study and selection since the 1,200 primary transformants were produced in 2005 resulted in the selection in July 2009 of a single Elite Event cabbage and a single cauliflower, judged the most appropriate for further development to registration.

THE CIMBAA REGISTERABILITY POSITION AT THE END OF PHASE III

At this stage in mid-2009, there was full proof of concept. Provisional acceptability of the material in India was demonstrated and had stakeholder support. The best plant lines were fully controlling the key target insects and performing much better than expected against *S. litura* and *H. armigera*. The Elite Events were chosen. There was reasonable clarity on the regulatory studies to be undertaken. DFID was prepared to consider funding these studies. CIMBAA was ready for Phase IV – the registration and licensing out of the developed material. The next steps were to:

- Finalize a commercialization agreement between the partners
- Undertake the regulatory studies (biosafety and nutrition etc) (Aug 2010 – Nov 2012)
- Submit the regulatory dossier in India
- Undertake the large scale agronomic field trials (April 2012- Feb 2014)
- Submit the dossier for biosafety approval:
  - In import jurisdictions – Europe, USA, Japan, Canada (April 2012)
  - In next tier growing countries – e.g.Philippines, Indonesia, Bangladesh, Pakistan, Thailand
- Obtain registration and release approval in India (2015?) requiring approval from:

- The Genetics Engineering Approval Committee (GEAC) for safety and efficacy
- The Ministry of Environment and Forests (and possibly the Ministry of Health) for environmental release

By this stage CIMBAA had utilized c. Euro 4 million in direct public funding and perhaps half as much again in public partner contributions. The private partner had expended at least a matching amount. Public sector donors were

- *India* – Australia-India Strategic Research Fund (2008-11)
- *Germany* – Eiselen Foundation grant to AVRDC (2006-08)
- *USA* – Program for Biosafety Systems (2005-08)
- *UK* – Department for International Development (2006-10)
- *Taiwan* – Council of Agriculture grant to AVRDC (2007-08)
- *Australia* – Australia-India Strategic Research Fund (2008-11)

OBSTACLES TO PROGRESS

Licensing/liability issues

In May 2009, AVRDC announced that it was not confident of its capacity to manage the licensing of CIMBAA material to private and public sector breeders. Having no experience in this area, and given the open-ended and uncertain nature of potential liability and the time and resources that might need to be spent in defending itself against accusations (justified or not), AVRDC believed that it was inappropriate to continue to take its intended role in the commercialisation process. AVRDC consequently withdrew as a partner in the CIMBAA collaboration. After taking stock of the new situation Cornell University also withdrew, citing a precedent in the Indian Bt eggplant development where they had been a major partner in the research phase but not been part of the commercialisation process.

A CIMBAA Steering Committee meeting in London in June 2009 came to the conclusion that Greenwich and Melbourne Universities did not have the capacity to take on the licensing role envisaged for AVRDC and, despite wide consultations, no other willing and capable public sector partner could be found. This left Nunhems as the potential commercializer / licenser of the CIMBAA material. This was, of course, a very major change in the CIMBAA concept. It was clear that if Nunhems was to take the liability and licensing function, they had to be free to commercialize the plants in such a way as to provide a commercial return to justify the investment and risk. Although Nunhems, in common with other private sector commercializers of GM crops, was clear that a large majority of the financial benefit from growing CIMBAA seeds would stay with the farmer, this shift had the potential to affect perceptions of the access to CIMBAA seeds by the poorer farmers. This had been the rationale for the CIMBAA partnership and the public sector support of it. DFID offered to support a study into potential mechanisms which would allow the commercialization process to go on in India while ensuring that the interests of poorer farmers were protected.

DFID commercialization options study

The DFID study reported in Feb 2010, having consulted widely amongst stakeholders, particularly in India. After consideration of other options, it proposed for consideration that, at least for a provisional period, the Bt seed price to small-scale farmers be supported through payments to, for example, the Seed Corporations in India, who would buy seed from Nunhems at a full commercial rate and sell them at a discount to farmers, although this raises issues of potential side-selling undermining the main market.

However, it was also clear that the feeling in India was that it was going to be important for the national acceptance of any GM food crop that it was not introduced as a monopoly of a single multi-national company. Sharing of the CIMBAA plant material with public breeders and Indian seed companies, preferably early enough in the breeding process to allow them to enter the market shortly after Nunhems, was seen as vital to obtaining regulatory and public approval and hence successful commercialization. This added a further pressure on the private partner. In addition to the commercialization and IP risk lying fully with the company rather than being shared with the public sector, there was now a presumption of early sharing of CIMBAA material with competitors and public breeders.

The agricultural biotechnology industry adopted a strict set of stewardship rules for the development, testing, registration, release and eventual withdrawal of biotechnology products and in particular GMOs (www.excellencethroughstewardship.org). The system requires close tracking / monitoring and reporting on the movement of all material in development and, critically, the tracking of seed material sold to farmers right through the supply chain. While commendably socially responsible, these requirements do have the effect of making it very difficult to share pre-breeding GMO material with any other organization or company, in particular prior to regulatory approval for commercial release. Bayer Crop Science is a signatory to the stewardship agreement, ruling out the requested sharing of CIMBAA material with public or smaller private vegetable seed breeders.

Liability concerns

Liability with respect to GM crops under development and post commercialization is unclear and varies across different regulatory environments (Boardi 2007) and is being quite largely guided by court cases, especially in North America. The Govt. of India has made it clear that the technology developer is regarded as holding full responsibility for the developed material at least prior to

regulatory approval and authorization for commercial release. Concern focused particularly around the liability for unauthorized release, both in India during the national agronomic trials process run by ICAR and the Agricultural Universities, and through the unauthorized movement of seeds into counties in which they were not approved and indeed which may have no approvals process in place. In addition to seeking biosafety and growing approval in India, CIMBAA planned to seek import approval in USA, Canada, Europe and Japan and cultivation approval in 'second tier' countries – such as Bangladesh and the Philippines. Synchronizing these approvals with the registration process in India was clearly going to be a very major challenge.

Regulatory challenges and the Bt eggplant experience

CIMBAA also had to consider the lack of progress with the Maharashtra Hybrid Seed Company (Mahyco) Cry1Ac Bt eggplants in India, which had also been donor supported, in particular by USAID. These plants had met all the regulatory requirements for approval and release, including the nation-wide agronomic testing of the transformed material by mid 2009. The company had made breeding material available to Agricultural Universities and indeed to Bangladesh and the Philippines. In late 2009 the decision on whether to release the Bt eggplants was passed to the Minister of Environment and Forests. He called for extensive public consultation over the following few months, ending in the decision in Feb 2010 to delay any release pending undefined further studies and setting up further review committees. The nature of these studies has not been defined subsequently nor have the committees reported. For CIMBAA this made the regulatory environment highly uncertain, given the commercial rule of thumb that ever year of delay in commercialisation of a new agricultural product knocks 10% of the net present value of the product. The fact that plants containing Cry1B protein have not yet been commercialised anywhere and that Bt cabbage would be the first GM food product to be consumed raw, might trigger additional unforeseen regulatory hurdles.

It became clear that the uncertainties in timelines and regulatory constraints made in extremely difficult to forecast the financial and human resources necessary to complete the program.

DISCONTINUATION

Taking all these factors into account, a meeting of the CIMBAA Steering Committee in May 2010 agreed that, in the current uncertain climate, there was little choice but to discontinue the development of the CIMBAA plant lines. In order to ensure that there was no unauthorized removal of plant material, all growing Bt plants in India were destroyed in mid May with the stakeholders and regulators informed immediately afterwards. Small quantities of seeds of the Elite lines, the hybrids created from them, plus back-up lines are now held only with Bayer Crop Science in Europe.

Next steps

The Indian Council for Agricultural Research has come forward with a willingness to take full responsibility for the CIMBAA material. However, it is private sector partner's understanding that the CIMBAA plant material cannot be made available to other developers as the responsibility for the GM plant material will be linked to the company as the developer of the plants, although there are other views on the transferability of ownership and with it liability. In any event ICAR and Bayer Crop Science are discussing the production of a slightly altered *cry1B/cry1C* gene construct to be wholly held in the public sector (ICAR) and used to transform important crop brassicas for eventual approval and release. The arrangement may include rights to transformation in kale, with ICAR interested in facilitating its use in Africa.

CONCLUSION

Technically the CIMBAA program has been a great success, with complete, and most likely sustainable, control of diamondback moth, cabbage cluster caterpillar and cabbage webworm, the three most important pest species across large parts of the world. It is likely that cabbage white butterfly would also be completely controlled in the open field. Although some damage from leaf worm and especially cotton bollworm, might be expected, these species are well controlled by their species-specific NPVs. No significant scientific obstacles to the successful registration of the CIMBAA material in India were seen. The Elite Events for commercialization were selected and stakeholder support was high. However, in the current climate of over-regulation of Bt crops and excessive liability regimes, it seems unlikely that public/private partnerships working on minor crops for the benefit of small farmers and consumers in poorer countries are going to be able to succeed. Using the re-engineered Bt construct which is expected to be produced for ICAR, will take at least four years of breeding, testing and selection before we again have high quality Bt lines ready for regulatory studies. There is already a proposal to stack RNAi control of aphids with the *cry1B/1C* Bt genes to produce the next generation plants.

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SESSION 2

Biology, ecology and behavior of diamondback moth and other crucifer pests

Studies on the biology and toxicity of newer insecticide molecules on cabbagehead caterpillar, *Crocidolomia binotalis* (Zeller) (Lepidoptera: Pyralidae) in India

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ABSTRACT

The biology, morphometrics, growth and development and susceptibility of cabbagehead caterpillar, *Crocidolomia binotalis* (Zeller) to new molecule insecticides were studied in laboratory conditions on cauliflower. The female moths laid their eggs in 7-12 masses of 15-80 eggs/mass on the abaxial surface of the leaves with incubation period of 3 days. The first, second, third, fourth and fifth instars of larvae lasted for 2.50, 2.50, 2.00, 2.00 and 2.00 days respectively. The prepupal and pupal periods lasted for 1.00 to 2.00 and 6.00 to 8.00 days, respectively. The longevity of male and female moths after oviposition was 2.50 and 1.50 days respectively. The total life cycle of male and female on cauliflower lasted for 32.50 and 31.50 days, respectively. The larva consumed on average 3181.21 mg cauliflower leaves during its growth period, of which 85.8 per cent was consumed during the last instar. Approximate digestibility (AD) and efficiency of conversion of ingested food (ECI) declined progressively to the lowest (14.60 and 9.16%) in the fifth instar, whereas efficiency of conversion of digested food (ECD) increased to the highest level (62.70%) in the last instar. On the basis of LC50 values, the order of toxicity was emamectin benzoate > spinosad > indoxacarb > azadirachtin > quinalphos with their corresponding LC50 values being 0.0221, 0.0323, 0.0763, 0.1791 and 1.6955 ppm, respectively.

INTRODUCTION

The importance of vegetables as protective foods and as suppliers of adequate quantities of vitamins, proteins, carbohydrates and minerals is well known. The per capita consumption of vegetables in India is only 135 g, although dieticians recommend 285 g for an adult per day for maintenance of health. The existing area under vegetable cultivation in India is around 6.7 million hectares with production of about 101.44 million tonnes.

Cauliflower, *Brassica oleracea* var. *botrytis*, is the most important cole vegetable crop grown in tropical and subtropical regions of India with an area of 2.79 lakh hectares and 44.44 lakh tonnes of production (Weinberger and Srinivasan 2009). Cauliflower remains a very important crop for smallholder farmers, providing income and nutrition and enabling small farms to remain financially viable, especially in the rapidly growing peri-urban farming sector. Cultivation of cauliflower is also associated with some insect pests. Among the various insect pests, the diamond back moth, *Plutella xylostella* (L.) is the most harmful and economically important pest of cauliflower in India. However, the cabbage head caterpillar, *Crocidolomia binotalis* (Zeller) (Lepidoptera: Pyralidae) which is the secondary pest of cauliflower, may become serious during the dry season. It also causes considerable yield loss to cruciferous crops like cauliflower, cabbage, radish and mustard.

The cauliflower leaves are skeletonised by the larvae of *C. binotalis* and they remain on the under surface of leaves in webs. The developed larvae feed on them voraciously and severe infestation results in entire defoliation of the plant. The quantitative consumption of food by an insect is the total response to feed as a whole with its nutrients, water contents and other physical as well as chemical components. Insect nutritional studies provide valuable information on the energy metabolism of biochemical components in insect body, and also can contribute key points to develop new management strategies. A larval density of 2-3 per plant could destroy the primordial tissues in cauliflower and prevent the establishment of young plants (Nagarkatti and Jayanth 1982). Information on the biology, morphometrics, susceptibility to new group of insecticides and quantity of food eaten by *C. binotalis* on cauliflower in India will be useful to develop effective pest management strategies in the future.

MATERIALS AND METHODS

Biology and morphometrics of *C. binotalis*

The biology and morphometrics of leaf webber, *C. binotalis* (Zeller) on cauliflower was studied during January to March, 2008 in the Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. Late instar larvae of *C. binotalis* were collected from cauliflower

fields of Thondamuthur village, Coimbatore. The larvae were reared in plastic buckets by providing fresh cauliflower leaves daily. The seedlings of food plants were transplanted in mud pots with a 50:50 mixture of sand and potting mixture in the greenhouse under natural day length, with a minimum daily temperature of 20°C to 24°C and a maximum of 30°C to 34°C. Plants were fertilized fortnightly, from one month after planting, and staggered planting was done at fortnightly intervals to obtain a regular supply of food for test insects. Fresh leaves taken from potted plants were used as food for the growing larvae.

The male and female adult moths obtained were released in cages for egg laying. A glass vial with 10 per cent sugar solution swabbed with cotton was kept inside the cage as adult food throughout the period of egg laying in which tender leaves of cauliflower were placed as egg laying substrate in conical flasks containing water. The leaves for egg laying were changed every 24 hours. After egg laying, eggs were transferred with fresh tender leaves to Petri plates at room temperature. A moistened filter paper was kept in each Petri plate to prevent the drying of leaves. The caterpillars were maintained in Petri plates up to second instar. Later they were transferred and reared in separate plastic buckets. Sterilized fine soil was provided to the last instar larvae for pupation and pupae were transferred to adult emergence cages to complete one generation.

Observations were recorded daily on the development of colour, size and duration of different instars of larvae. The body length, width of head capsule of first instar (ten larvae) and eggs were measured using ocular micrometer, while second instar to fourth instar, pre-pupal and pupal morphometrics data were recorded using a standard graph paper method. The duration of prepupal, pupal stages, changes in colour were also recorded and analysed statistically (Gomez and Gomez 1984). Fecundity studies were made by releasing ten individual pairs of freshly emerged adults which were kept separately in cages containing fresh tender leaves placed in conical flask with water. Observations regarding pre-mating, mating, pre-oviposition and post-oviposition periods for females and adult longevity, body length, width, wing expansion and sex ratio for both male and female were also recorded.

Quantitative intake of food and nutritional indices studies

Newly hatched larvae of C. *binotalis* were kept in groups of 20 in Petri plates. When they reached fourth instar stage, they were segregated and housed singly for further studies. The larvae were provided with a known quantity of food (cauliflower leaves) daily at noon until pupation. Daily records of weight of larva, food offered, food left uneaten and excrement produced was maintained. Various nutritional indices, i.e. consumption index (CI), approximate digestibility (AD), growth rate (GR), efficiency of conversion of ingested food (ECI) and efficiency of conversion of digested food (ECD) were calculated as per procedure (or) method given by Waldbauer (1968) on fresh weight basis.

Culturing of C. *binotalis*

Egg masses of *C. binotalis* were collected from the cauliflower field and kept in Petri plates for hatching; the hatched larvae were transferred using a fine camel hair brush to tender whole cauliflower leaves, with petioles inserted into conical flasks of water. Second instar larvae were transferred to plastic buckets and covered with muslin cloth. Food material was changed once every two days for early instars, and daily for late instars. Sterilized fine soil was provided to the last instar larvae for pupation and pupae were transferred to adult emergence cage. Ten percent sugar solution served as adult food and fresh cauliflower leaves were provided for egg laying. Eggs with leaves were kept in Petri plates for hatching and the procedure was continued.

Toxicity of new molecule insecticides

Third instar larvae were used for bioassay. Leaf discs of 6 cm diameter were cut on either side of midrib of leaf. These discs were dipped in spinosad 2.5 SC (Success), indoxacarb 14.5 SC (Avant), emamectin benzoate 5 SG (Proclaim), Azadiractin 0.5 EC (Econeem) and quinalphos 25 EC (Ekalux) respectively for about 30 seconds. Leaf discs were shade dried and transferred into 10 cm x 6 cm plastic containers. Leaf discs were placed at a slant to enable the larvae to move on either side. Ten larvae were released in each container with five replications. Leaf discs dipped in distilled water served as control (Tabashnik and Cushing 1987). Mortality was recorded 24 hours after treatment and data were subjected to probit analysis (Finney 1971).

RESULTS

Egg

The adult female moths laid their eggs in batches on the abaxial surface near the base of the leaf. Initially the eggs were light green in colour. Before hatching, the colour turned to brown. The eggs were flattened, circular, laid in overlapping masses of 15 to 80 eggs with an average of 47.5 egg/batch and were packed neatly like roof tiles. The average length and breadth of the egg mass were measured at 3.63 and 3.75 mm, respectively. The length of single eggs ranged from 0.27 to 0.38 mm, while the breadth was 0.24 to 0.38 mm and incubation period was 3.0 days.

Larva

The newly hatched caterpillars have black heads and light green bodies with dark spots. The older caterpillars have pale whitish longitudinal stripes, three dorsally, one on each lateral side. Between the dorsal and lateral stripes, black round spots are present. These are the bases of long hairs on the body. The larvae undergo five larval instars with a mean larval period lasting 11.0 days. The mean duration of first, second, third, fourth and fifth instars was 2.50 (2-3 days), 2.50 (2-3 days), 2.00, 2.00 and 2.00 days, respectively (Table. 1). The head capsule sizes of the above instars measured about 0.27, 0.46,

0.84, 1.40 and 1.69 mm, respectively (Table 2). The neonate larvae were gregarious on the abaxial surface and, for up to 5 days after hatching, fed by scraping the leaf resulting in a papery appearance. The medium size larvae fed on the underside of the leaf without eating through the uppermost leaf layer and created window-like damage. In the later stage, the larvae caused defoliation, entered into the head of the cauliflower and made tunnels, which were covered by fine silken webs with rotting of crops.

Table 1. Duration of different stages in the life cycle of *Crocidolomia binotalis* on Cauliflower

S. No.	Particulars/Stage	Duration (Days)		
		Minimum	Maximum	Mean ± SEM*
1.	Egg period	2.0	4.0	3.0 ± 0.06
2.	Larval period			
	I instar	2.00	3.00	2.50 ± 0.040
	II instar	2.00	3.00	2.50 ± 0.043
	III instar	1.50	2.50	2.00 ± 0.025
	IV instar	1.50	2.50	2.00 ± 0.020
	V instar	1.50	2.50	2.00 ± 0.023
	Total larval period	8.50	13.50	11.00 ± 0.047
3.	Pre-pupal period	1.00	2.00	1.50 ± 0.023
4.	Pupal period	6.00	8.00	7.00 ± 0.128
5.	Pre-oviposition period	1.00	2.00	1.50 ± 0.022
6.	Oviposition period	5.00	7.00	6.00 ± 0.145
7.	Adult longevity			
	Male	2.00	3.00	2.50 ± 0.237
	Female	1.00	2.00	1.50 ± 0.204
8.	Total duration of life cycle (Egg to adult longevity)			
	Male	26.00	40.00	32.50 ± 0.962
	Female	25.00	39.00	31.50 ± 0.946
9.	Adult emergence (%)	62.27	72.45	67.36 ± 1.462
	Male	27.98	32.55	30.26 ± 0.653
	Female	34.30	39.90	37.10 ± 0.801
	Sex ratio			1 : 1.23

Pupa

When the larvae reached full growth, they burrowed into the soil near the base of the plant and pupated. The freshly formed pupae were yellowish-brown, which later became dark brown in colour. The average size of the pupa was 10.0 mm in length with 3.0 mm width in males and 12.0 mm in length with 4.0 mm width in females. The pupal period ranged from 6 to 8 days.

Adult

The female moths emerged about one day before the males. Adults have black thoraxes and reddish brown abdomens. Females bear a curved ovipositor and were generally larger than males. During the day they hide under the cauliflower leaves and when disturbed, they were able to fly for short distances. In both males and females, there were considerable variations in delineation of markings on the cream background of the forewings. Males have greater delineation and were easily recognized by a tuft of dark hairs at the anterior margin of the forewings, which the females lacked (Figure 1). The adult wing span was 21.5 and 19.5 mm in males and females, respectively. Males have larger bodies (12.5 mm) than females (10.0 mm). But, visually, females have larger abdomens than males. On an average, male and female moths survived for 10.0 and 9.0 days. The total lifecycle of males and females lasted for 32.5 and 31.5 days. The mean pre-oviposition, oviposition and adult longevity after oviposition for a female were 1.5, 6.0 and 1.5 days, respectively.

Table 2. Morphometrics of *C. binotalis* on cauliflower

S. No.	Particulars/Stage	Measurements		
		Minimum	Maximum	Mean ± SEM
1.	Total number of egg masses / female	7.00	12.00	10.00 ± 0.29
2.	Number of eggs / mass	15.00	80.00	47.50 ± 1.37
3.	Egg mass			
	Length (mm)	3.02	4.04	3.63 ± 0.105
	Breadth (mm)	1.60	3.90	3.75 ± 0.108
4.	Egg			
	Length (mm)	0.27	0.38	0.34 ± 0.009
	Breadth (mm)	0.24	0.38	0.28 ± 0.008
5.	Larval head capsule width (mm)			
	I instar	0.27	0.29	0.27 ± 0.008
	II instar	0.43	0.47	0.46 ± 0.013
	III instar	0.79	0.86	0.84 ± 0.024
	IV instar	1.29	1.48	1.40 ± 0.040
	V instar	1.62	1.76	1.69 ± 0.036
6.	Pupal weight (g)			
	Male	0.80	1.20	1.00 ± 0.081
	Female	1.00	1.40	1.20 ± 0.109
7.	Pupal length (mm)			
	Male	1.81	2.01	1.91 ± 0.055
	Female	2.08	2.30	2.19 ± 0.063
8.	Pupal breadth (mm)			
	Male	0.58	0.65	0.62 ± 0.018
	Female	0.63	0.69	0.66 ± 0.019
9.	Adult wingspan (mm)			
	Male	20.43	22.58	21.50 ± .621
	Female	18.53	20.48	19.50 ± .563
10.	Adult body length (mm)			
	Male	11.88	13.13	12.50 ±0.361
	Female	9.50	10.50	10.00 ± .289

Mating, oviposition and fecundity

The sex ratio of cabbage head caterpillar was 1:1.23. Moths mated after 1-2 days from emergence. Mating occurred during night hours and oviposition took place one day after mating during the night. The oviposition period lasted for 5-7 days. A single female was able to deposit 7 to 12 egg clusters with a total number of 15-80 eggs per cluster; average fecundity of a female was 475 eggs.

Quantitative intake and utilization of food

The present study also revealed that the *C. binotalis* larva consumed, on average, 3.80, 18.09, 64.22, 364.13 and 2730.97 mg of fresh cauliflower leaves, respectively, in five successive instars, thereby consuming a total amount of 3181.21 mg leaves during its entire larval life period (Table 3). It was observed that the *C. binotalis* caterpillar fed gregariously in the first three instars by scraping and making holes in the leaves, whereas the later two instars scattered and nibbled at the leaf margins, eating the leaf voraciously, leaving behind only the hard midrib and veins intact. The consumption index (CI) and growth rate (GR) calculated on fresh weight basis was highest (5.46 and 0.47) in the fifth instar. Approximate digestibility (AD), was maximum in the second instar (53.79%) and minimum in the fifth instar (14.60%). Fresh weight efficiency of conversion of ingested food (ECI) in *C. binotalis* decreased from 29.13 in the second to 9.16 percent in the fifth instar. Efficiency of conversion of digested food (ECD) on the other hand increased progressively in the successive instars and was maximum in the fifth instar.

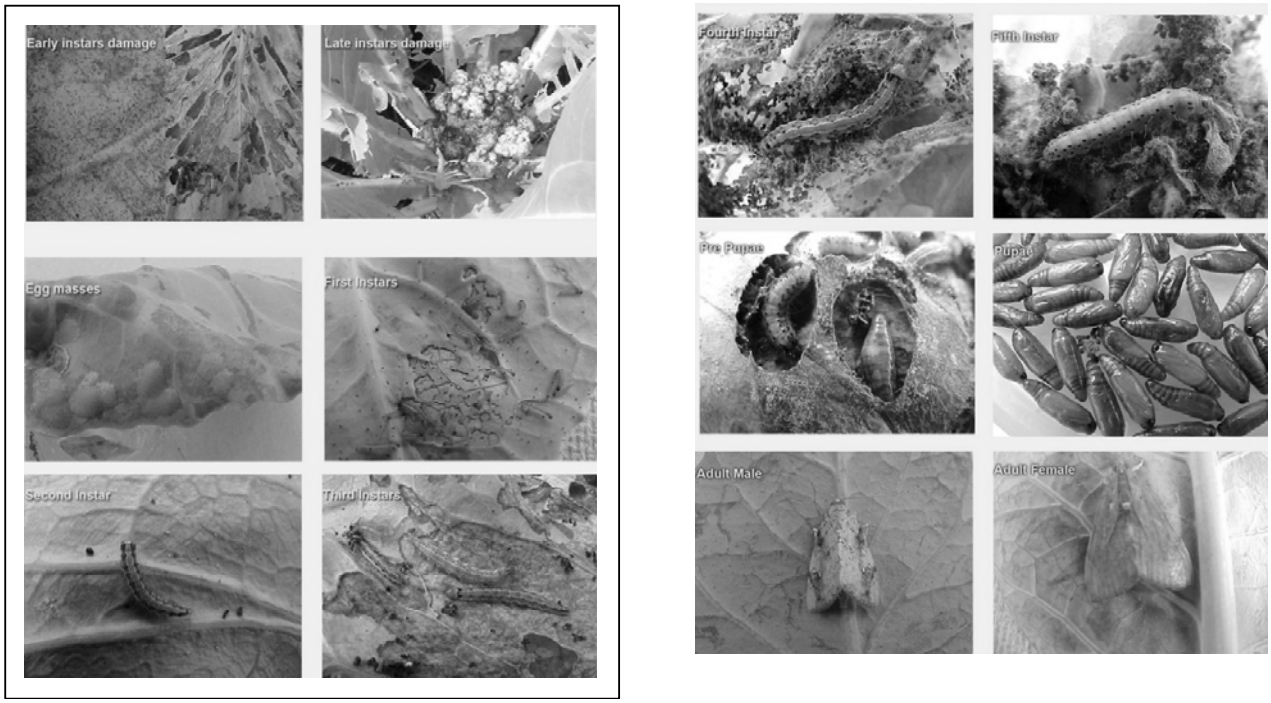

Figure 1. Biology of *Crocidolomia binotalis* on Cauliflower

Table 3. Nutritional indices and growth rate in *C. binotalis* on cauliflower leaves

Instar	Fresh weight of food consumed (mg/larva)	Weight gained/ larva (mg)	Faecal matter/ larva (mg)	Duration of feeding (Days)	GR	CI	AD	ECI (%)	ECD (%)
I	3.80	0.95	1.85	2.5	0.22	1.60	51.31	25.0	48.72
II	18.09	5.27	8.36	2.5	0.28	1.37	53.79	29.13	54.16
III	64.22	9.41	35.53	2.0	0.22	3.47	44.67	14.65	32.79
IV	364.13	51.66	279.98	2.0	0.37	3.52	23.11	14.19	61.39
V	2730.97	250.03	2332.23	2.0	0.47	5.46	14.60	9.16	62.70
Total larval period	3181.21	317.32	2657.95	11.0		1.40	17.04	8.37	23.61

Table 4. Susceptibility of *C. binotalis* to newer molecules of insecticides

Insecticides	No. of larvae	X^2 (n-2)	Slope ± SE	LC_{50} (ppm)	Fiducial limits (ppm)		LC_{90} (ppm	Fiducial limits (ppm)	
					Lower	Upper		Lower	Upper
Spinosad 2.5 SC	350	0.113	1.195	0.0323	0.023	0.065	0.411	0.193	0.876
Indoxacarb 14.5 SC	350	0.643	1.259	0.0763	0.052	0.113	1.547	0.449	0.943
Emamectin benzoate 5 SG	350	0.091	1.587	0.0221	0.015	0.032	0.241	0.118	0.490
Azadirachtin 0.1 EC	350	0.205	1.910	0.1791	0.166	0.192	0.342	0.314	0.380
Quinalphos 25 EC	350	0.715	2.022	1.6955	1.590	1.884	2.529	2.374	2.801

On the basis of LC_{50} values, the study also reveals the order of toxicity of newer molecule insecticides was emamectin benzoate (Proclaim) > spinosad (Success)> indoxacarb (Avaunt)> azadirachtin (Econeem) > quinalphos (Ecolux) with their corresponding LC_{50} values being 0.0221, 0.0323, 0.0763, 0.1791 and 1.6955 ppm respectively. LC_{95} values were 0.241, 0.411, 1.547, 0.342 and 2.529 ppm for emamectin benzoate, spinosad, indoxacarb, azadirachtin and quinalphos. Among the chemicals tested, emamectin benzoate was found to be most toxic to *C. binotalis* (Table 4).

DISCUSSION

The cabbagehead caterpillar, *C. binotalis* is a secondary pest of cauliflower and causes economic losses during the dry season.

The present studies indicated that female moths laid their eggs in batches on the lower side near the base of the leaf and average number of eggs laid by a gravid female was 47.50 with incubation period of 3 days. The present study also recorded five larval instars with mean total larval period of 11 days. The duration of first and second successive instars was 2.5 days; third, fourth and fifth instars were 2.0 days. Pupal period lasted for 6.0 -8.0 days. Average longevity of adult moth was 10.0 days and fecundity was 7 to 12 egg clusters with a mean of 475 eggs / female. The results of the present study are in agreement with the findings of Othman (1982) and Harcourt (1962). They have reported similar results with variations in duration of egg, larval, pupal periods and adult longevity. Such variations in the duration of different life stages may be due to geographic and climatic conditions.

The results of the morphometrics are in agreement with findings of Othman (1982) and Harcourt (1962). Studies on food consumption and nutritional indices revealed that *C. binotalis* larva consumed a total amount of 318.21 mg cauliflower leaves during its entire larval period, increasing its nutritional needs as it grew. Food consumption increased towards the end of each larval instar. This confirmed the findings of Sharma et al. (1999) who reported that a direct correlation was observed between food consumption and mean larval weight in cabbage white butterfly, *Pieris brassicae* (L.) on cauliflower.

Present studies also clearly indicated that the food consumption per larva as well as per gram body weight increased many-fold in the last instar, which alone accounted for 85.8% of total consumption during the entire larval period. Higher growth rate indicates higher utilization of ingested food for growth. Both growth rate and weight gain in *C. binotalis* indicated that the maximum utilization of food for tissue synthesis took place in the fifth instar. The results of the studies are in agreement with findings of Smith et al. (1986) and Sharma et al. (1999). They have recorded that half of the feeding and body weight gained occurred during the last two days of larval growth in soybean looper and *P. brassicae* in cauliflower.

The consumption index and growth rate was highest in fifth instar; approximate digestibility (AD) was maximum in the second instar (53.79%) and minimum in the fifth instar (14.60%), ostensibly because the food ingested by fifty instar larvae contained a non-digestible cellulosic component in much higher proportion and quality. This resulted in the production of increased excrement, i.e. 2332.23 mg in the fifth instar. Efficiency of conversion of ingested food (ECI) in *C. binotalis* decreased from 29.13 in the second to 9.16 per cent in the fifth instar. Efficiency of conversion of digested food (ECD) on the other hand increased progressively in the successive instars and was maximum in the fifth instar. ECI tends to decrease with increasing age in most of the insects, partly due to a concomitant decline in AD. An increase in ECD with declining AD and ECI can be attributed to the fact that the insect utilized more food for assimilation in the tissue in the later stages as manifested by increase in the weight of the insect. This result is in agreement with the findings of Reynolds et al. (1985) and Sharma et al. (1999). They have reported that the efficiency of diet utilization increased with increased water content in the food. The increase in ECD with decline of AD and ECI was attributed to the fact that the insect utilizes more food for assimilation in the tissue in the later stage to increase body weight.

Among the new molecules evaluated, emamectin benzoate ranks first in efficacy against *C. binotalis*. The results of the present study are in agreement with the findings of Suganyakanna et al. (2005) and Mohite and Patil (2005) against *P. xylostella* in cabbage.

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Natural mortality of *Plutella xylostella* L. (Lepidoptera: Plutellidae) and *Crocidolomia pavonana* F. (Lepidoptera: Crambidae) in commercial cabbage crops in the highlands of West Java, Indonesia

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ABSTRACT
A combination of life-table and natural enemy exclusion techniques was used to investigate the impact of natural enemies on *P. xylostella* and *C. pavonana* populations in commercial cabbage crops at Lembang, West Java in 2008 and 2009. In two of the three studies conducted, survivorship of *P. xylostella* was significantly increased by the exclusion of natural enemies, indicating that the endemic natural enemy complex has the capacity to suppress the pest population. In these studies the major causes of *P. xylostella* mortality in cohorts exposed to natural enemies were egg parasitism by unidentified Trichogrammatidae, disappearance of first, second and third instar larvae and larval parasitism by *Diadegma semiclausum* Hellén (Hymenoptera: Ichneumonidae). In a third experiment a heavy rainfall event close to the time of *P. xylostella* egg hatch resulted in significant mortality in both experimental cohorts. Survivorship of *C. pavonana* was also significantly increased by the exclusion of natural enemies from cabbage plants and the major causes of mortality in natural enemy-exposed cohorts were egg disappearance, blackened eggs which failed to hatch and the disappearance of neonate and second instar larvae. Visual sampling of plants and pitfall trapping during the experiments showed that the major arthropod predators present in the crops were Aranae, Staphylinidae and Formicidae. The commercial crops within which the studies were conducted were regularly treated with insecticides (a range of synthetic pyrethroids, spinosad, emmamectin and *Bacillus thuringiensis*) and while predator populations were significantly depressed following the application of pyrethroids, other insecticides had only a minor impact on predator abundance. The results are considered within the context of the small-scale mixed farming agro-ecosystem and the factors which might have contributed to the highly effective nature of the arthropod natural enemy complex in the insecticide intensive cropping system are considered.

Keywords
Predation; parasitism; *Diadegma semiclausum;* biological control; integrated pest management.

INTRODUCTION
Brassicaceous crops are amongst the most important vegetable crops in Indonesia. Most are cultivated in Central and West Java where they accounted for 26% and 22% of the 1.3 million tons produced nationally in 2008 (BPS 2010). This production ranks Indonesia as the 7th largest producer of *Brassica* crops in the world (FAOSTAT 2010) but yields are poor and harvests of only 21.4 kg/ha rank Indonesia only 66th in the world in terms of productivity (FAOSTAT 2010).

Insect pests present a severe constraint to *Brassica* crop production in the highlands of Java and the diamondback moth (DBM) (*Plutella xylostella* L. Lepidoptera: Plutellidae) and cabbage head caterpillar *(Crocidolomia pavonana* F. (Lepidoptera: Crambidae) are the most destructive and can cause total crop loss if not controlled (Sastrosiswojo and Sastrodiharjo 1986). *Plutella xylostella* is renowned for its ability to evolve resistance to all classes of insecticides which have been used against it and no insecticide alone offers a sustainable solution to manage this pest. The continued use of insecticides against *P. xylostella* has compromised biological control programmes based on the release of *Diadegma semiclausum* Hellén (Hymenoptera: Ichneumonidae) in highland regions of the tropics but the parasitoid has been reported to be well established in the highland cabbage growing areas of Indonesia (Sastrosiswojo and Sastrodiharjo 1986). Biological control of *P. xylostella* is often further compromised by the use of insecticides to control co-occurring *C. pavonana,* and in Indonesia, farmers still regularly apply broad spectrum insecticides for the control of *C. pavonana* and other cabbage pests. This maintains high selection pressures for the evolution of insecticide

resistance in *P. xylostella* and can trigger population outbreaks. In contrast to *P. xylostella*, *C. pavonana* has not developed resistance to the insecticides used against it; however, its natural enemy fauna is poorly understood and no parasitoids which could be employed for its effective biological control are known.

Despite some pioneering work on the ecology of *C. pavonana* that was conducted in Lembang in the 1980s (Sastrosiswojo and Setiawati 1992), little research has since been conducted on the ecology of *C. pavonana* in Indonesia. In order to develop sustainable pest management programs for *Brassica* crops, an effective biological control program for *P. xylostella* must be integrated with effective control measures for *C. pavonana* (e.g. selective insecticides, manipulation of endemic predator complexes, trap crops and other means of host plant manipulation). Biological control can form the foundation for IPM strategies for the management of *P. xylostella* and other crucifer pests (e.g. Furlong *et al*. 2004a, Furlong *et al*. 2008a) but in order to take this approach, a detailed understanding of pest and natural enemy ecology is required (Furlong and Zalucki 2007; Furlong *et al.* 2008b).

In this study, experiments were conducted in commercial head cabbage crops in 2008 and 2009. Natural enemy exclusion techniques were used to measure the impact of endemic natural enemies on experimental populations of *P. xylostella* and *C. pavonana* in order to begin to understand the endemic natural enemy complex available for incorporation into IPM programmes. The experiments were complimented by simultaneous sampling for both insect pests and their natural enemies.

MATERIALS AND METHODS

Plants, insects and field sites

Head cabbage (*Brassica oleracea* cv Green Coronet) was chosen as a study crop as it is widely grown in highland regions of West Java. Potted plants were grown from seed in 15 cm diameter pots in a screen house and reared to the 8-10 leaf stage before use for insect rearing or the 6-8 leaf stage for using in natural enemy impact studies.

Laboratory cultures of *P. xylostella* and *C. pavonana* were established in 2008 and 2009 from larvae collected from cabbage crops at the Indonesian Vegetable Research Institute (IVEGRI), Lembang, West Java (6^{o} 49'S 107^{o} 38'E). Similar culturing protocols were followed in both species. Larvae were reared in ventilated plastic containers (30 cm x 20 cm x 10 cm) and fed on fresh cabbage leaves daily. When pupae developed they were collected and placed in a Petri dish (9 cm diameter) which was then transferred to the base of a large (1 m x 0.75 m x 0.75 m) screened oviposition cage within a screen house. Upon eclosion adult moths were supplied with fresh honey solution (10% v/v) as a food source and young (8-10 leaf stage) potted cabbage plants on which to oviposit. Oviposition plants were changed daily and larvae of both species were allowed to develop to the second instar on these plants before they were transferred to plastic rearing boxes.

Field experiments were conducted in commercial head cabbage crops in Lembang. In 2008 cabbage seedlings were transplanted to the field on 10 January. The field (≈0.14 ha) consisted of 25 raised beds (40 m long) spaced 0.75 m apart. Each bed contained two parallel rows of plants; rows within a bed and adjacent plants within a row were spaced 0.6 m apart. In 2009 cabbage seedlings were transplanted to the field on March 5. The field (≈0.19 ha) consisted of 59 slightly raised beds (27.5 m long) spaced 0.5 m apart. Each bed contained 3 parallel rows of cabbage plants; rows within a bed were 0.35 m apart and plants in the middle row were offset from the plants in the 2 outer rows by 0.175 m, plants within a row were spaced 0.6 m apart. In both crops, agronomic and pest management practices were under the complete control of the farmer and detailed records of all insecticides applied were maintained. Comprehensive records of the crops grown in the immediate vicinity of the experimental crops were not kept but in both study years some proximate fields contained other *Brassica* crops which were more mature than the experimental crops and others were planted with *Brassica* seedlings following the establishment of the experimental crop.

In both 2008 and 2009 daily maximum and minimum temperatures and daily rainfall were recorded at the IVEGRI weather station, located ≈500 m from the experimental sites.

Assessment of natural enemy impact on *P. xylostella* and *C. pavonana* populations

The impact of natural enemies on experimental populations of *P. xylostella* and *C. pavonana* was evaluated by comparing survivorship of cohorts of each insect on cabbage plants caged to exclude arthropod natural enemies with survivorship of cohorts on plants to which these natural enemies had access. Cages consisted of a cylindrical central wire frame (45 cm diameter by 45 cm height) covered by a fine nylon mesh sleeve (gauge ≈1 mm^{2}). Modifications to the mesh sleeve allowed the construction of cages which completely excluded natural enemies (the mesh was buried under experimental plants and completely covered the top and sides of the wire frame) and the construction of cages to which natural enemies had access (the mesh only covered the upper parts of the wire frame but the 15 cm immediately above ground level allowed access of predators and parasitoids to the cage) but which created the same micro-climatic conditions as the natural enemy exclusion cages (Furlong *et al*. 2004b).

For the *P. xylostella* experiments eggs were collected by placing four mated female moths (2-3 days post eclosion) in a Petri dish (9 cm diameter) with a small Chinese cabbage leaf disc (≈4 cm diameter) for 24 h under conditions of ambient temperature and humidity. Eggs laid on the leaf disc were then counted using a hand lens

(x10 magnification) and excess eggs removed from each leaf disc so that each supported 20 eggs which were < 24 h old. Egg-laden leaf discs were then taken to the field and each was carefully pinned to the lower surface of the fifth leaf on 16 experimental potted cabbage plants (6-8 leaf stage). For the *C. pavonana* experiments, egg masses (<24 h old) were carefully removed from plants placed in an oviposition cage by cutting around the adjacent leaf tissue. They were then examined under a microscope and the number of eggs in each determined. When sixteen egg masses of approximately similar size had been collected, each was allocated to one of the cage treatments. The mean number of eggs in the eight egg masses allocated to the open cage treatments was 33.5 (±4.6) and the mean number of eggs in the egg masses allocated to the exclusion cage treatment was 31.0 (±3.6). Eggs were pinned to the lower surface of the fifth leaf on experimental potted cabbage plants as previously described. Experimental plants were then buried in the ground within open or natural enemy exclusion cages.

In all experiments cages were arranged in a stratified random manner within the crop fields with a minimum distance of 5m separating adjacent cages. In 2008, a single *P. xylostella* experiment was conducted. Three separate experiments were conducted in 2009; two experiments assessed the impact of natural enemies on *P. xylostella*, while a third experiment assessed the impact of natural enemies on *C. pavonana*. The three experiments were run consecutively in the same plot. In all the *P. xylostella* experiments, all caged plants were examined 2-3 times per week and the number and developmental stage of the larvae were recorded. The experiments were terminated when the majority of individuals in the cohort reached pre-pupal stage. Cabbage plants were then cut at the base and placed into a labeled plastic bag together with any immature *P. xylostella* recovered after close examination of the plant pot and the interior of the cage. The bags were sealed and transported to the laboratory. All *P. xylostella* larvae and pupae were collected from each plant and then reared together in Petri dishes (9 cm diameter) under ambient conditions of temperature and humidity. The number of immature *P. xylostella* recovered from each plant and post collection mortality and parasitism rates were recorded. In the *C. pavonana* experiment, all caged plants were examined 2-3 times per week and the number and developmental stage of the larvae were recorded. However, this experiment was terminated when the larvae in the experimental cohorts reached the 3rd instar. Plants and larvae were then collected in the same manner as described for the *P. xylostella* experiments. The number of insects on each plant was then determined in the laboratory but the insects were not reared further.

Crop monitoring and insecticidal interventions

During the experiments the commercial head cabbage crops were monitored every 3 days and the number and developmental stage of pest insects and beneficial arthropods on 30 randomly selected plants were recorded. Epigeal predators within experimental crops were monitored using pitfall traps. Traps were made from plastic cups (8cm diameter) filled with 100 ml of detergent solution (1% vol/vol) and covered with a plastic disc (15 cm diameter) supported 3 cm above the lip of the cup. The pitfall traps were carefully constructed so that the lip of the trap was level with the soil surface and care was taken to cause minimal disturbance to the surrounding soil. On each examination date the contents of each trap were poured into a labeled screw top plastic beaker and then transported to the laboratory where predatory arthropods were sorted and identified to family. All insecticide applications made by the farmer were also recorded (when insecticides were applied the cages were covered with heavy duty plastic bags which were removed as soon as possible after insecticide treatment to prevent overheating).

Statistical analysis

Proportional recovery and survival rates of *P. xylostella* and *C. pavonana* cohorts from the different cage treatments were subject to arc-sine transformation prior to t-tests.

RESULTS AND DISCUSSION

Assessment of natural enemy impact on *P. xylostella* and *C. pavonana* populations

In the 2008 experiment the proportion of the *P. xylostella* cohort recovered from plants within natural enemy exclusion cages (0.69 (±0.04)) was significantly greater than the proportion recovered from plants to which natural enemies had access (0.13 (±0.04)) ($t=7.448$ $df=14$, $P<0.0001$; Fig 1A). In the first experiment conducted in 2009, recovery of *P. xylostella* was low in both treatments (0.19 (±0.01) from cages which excluded natural enemies and 0.05 (±0.01) from cages which allowed natural enemy access) and there was no significant difference in the recovery rates between treatments ($t=1.90$, $df=14$, $P=0.08$; Fig 1B). In the second experiment conducted in 2009 the proportion of the *P. xylostella* cohort recovered from natural enemy exclusion cages (0.49 (±0.06)) was significantly greater than the proportion of the cohort recovered from cages to which natural enemies had access (0.08 (±0.01) ($t=7.347$, $df=14$; $P<0.0001$; Fig 1C).

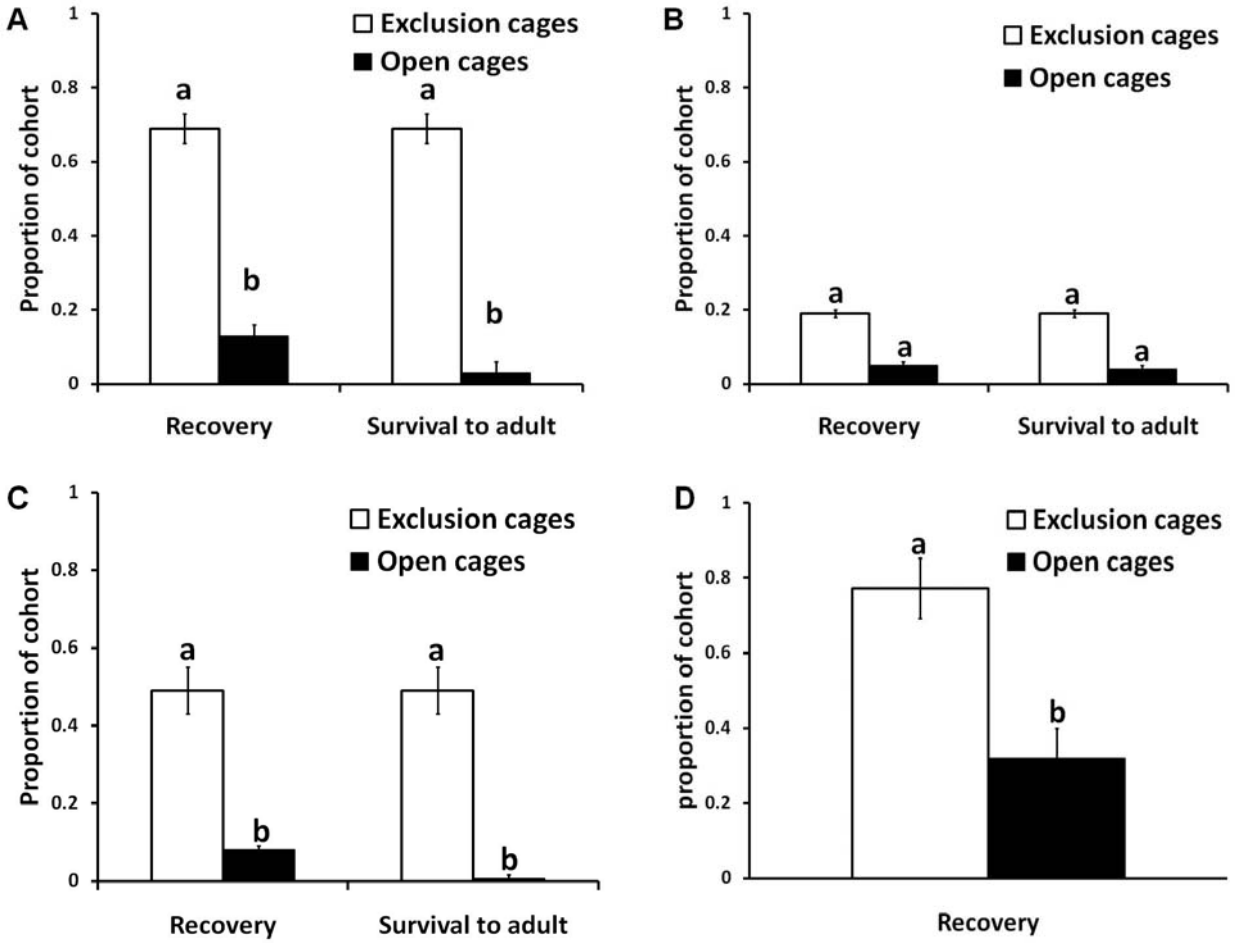

Figure 1. Mean (±SE) recovery and mean (±SE) survival of A) *P. xylostella* cohorts in the 2008 experiment; B) *P. xylostella* cohorts in the first experiment in 2009; C) *P. xylostella* cohorts in the second experiment in 2009 D) *C. pavonana* cohorts in 2009. For each experiment recovery and survival rates between treatments which are marked with a different letter are significantly different (t-test, *P*<0.05)

Similarly survivorship to adults was significantly affected by cage treatment in 2008 (survival in exclusion and natural enemy access cages=0.69 (±0.04) and 0.03 (±0.03) respectively (t= 13.31, df=14, *P*<0.0001; Fig 1A) and in the second experiment in 2009 (survival in exclusion and natural enemy access cages=0.49 (±0.06) and 0.006 (±0.0003) respectively (t=10.44, df=14, *P*<0.0001; Fig 1C) but not in the first experiment in 2009 (survival in exclusion and natural enemy access cages=0.19 (±0.01) and 0.04 (±0.01) respectively (t= 212, df=14, *P*= 0.052; Fig 1B). In the *C. pavonana* experiment in 2009 the proportion of the cohort reaching the third instar in the natural enemy exclusion treatment (0.77 (±0.05)) was significantly greater than the proportion reaching the third instar in the natural enemy access treatment (0.33 (±0.07)) (t= 4.036, df= 13, P= 0.001 Fig 1D).

Frequent sampling of the experimental insects during each experiment allowed construction of lifetables for cohorts developing simultaneously in the presence or absence of natural enemies (Fig. 2A-D).

In 2008 parasitism by *D. semiclausum* was the single greatest mortality factor in insects exposed to natural enemies, followed by disappearance of neonate larvae and the disappearance of second and third instar larvae respectively (Fig 2A). Unidentified Trichogrammatidae caused some mortality in eggs exposed to natural enemies (Fig 2A). In the absence of natural enemies the net reproductive rate (Ro) of the cohort was 41.7 while the presence of endemic natural enemies reduced Ro to only 1.5 (Fig 2A), indicating that the natural enemy complex reduced the rate of *P. xylostella* population growth. In the first experiment in 2009 *P. xylostella* survivorship in both experimental cohorts was low (Fig. 1B) and by far the single the greatest mortality factor in both experimental cohorts was egg disappearance (Fig 1B). Placement of eggs on experimental plants in this experiment coincided with sustained heavy rainfall and it is likely that this abiotic mortality factor is responsible for the high levels of egg (and possibly neonate) disappearance observed. In the second experiment conducted in 2009 egg disappearance in *P. xylostella* cohorts in both cage treatments was recorded and heavy rainfall is again the most likely reason (Fig 2C).

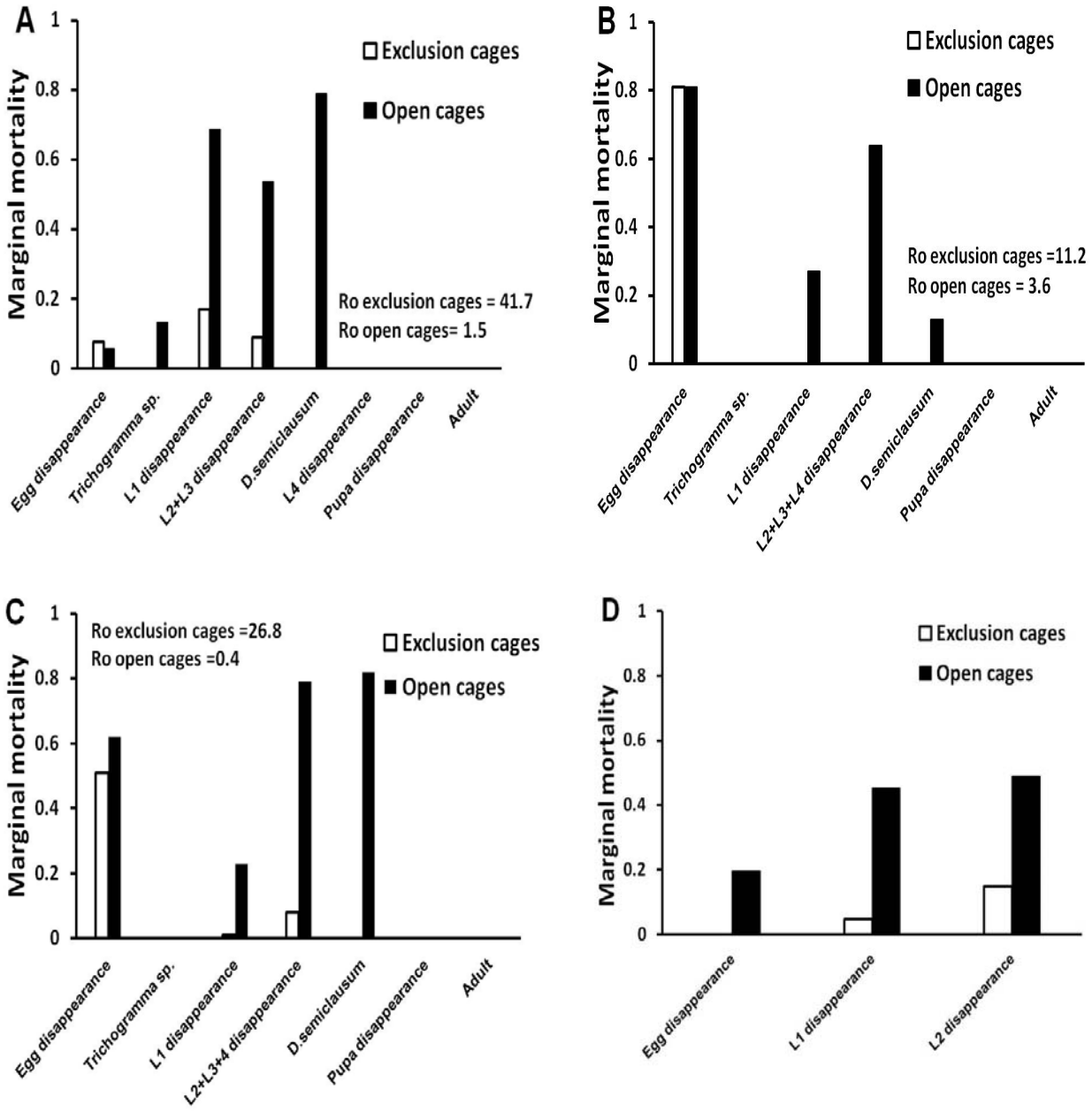

Figure 2. Lifetables for insect cohorts developing simultaneously in the presence (open cages) or absence (exclusion cages) of natural enemies. A) *P. xylostella* cohorts in the 2008 experiment; B) *P. xylostella* cohorts in the first experiment in 2009; C) *P. xylostella* cohorts in the second experiment in 2009; D) *C. pavonana* cohorts in 2009. The net reproductive rate (Ro) of each P. *xylostella* cohort in each experiment is also shown.

However, the greatest mortality factor impacting on insects exposed to natural enemies was parasitism by *D. semiclausum* followed by second, third and fourth instar disappearance (Fig 2C). In the absence of natural enemies the net reproductive rate (Ro) of the cohort was 26.8 while the presence of endemic natural enemies reduced Ro to 0.4 (Fig 2C), indicating that the natural enemy complex caused the *P. xylostella* population to decline between successive generations.

In the *C. pavonana* experiment egg masses exposed to natural enemies suffered greater marginal losses than those in exclusion cages and several eggs within egg masses became blackened; however, no parasitoids emerged from these eggs (Fig 2D). Similarly neonate and second instar larvae disappearance was greater in cages to which natural enemies had access than in natural enemy exclusion cages, indicating that generalist predators attacked immature *C. pavonana*.

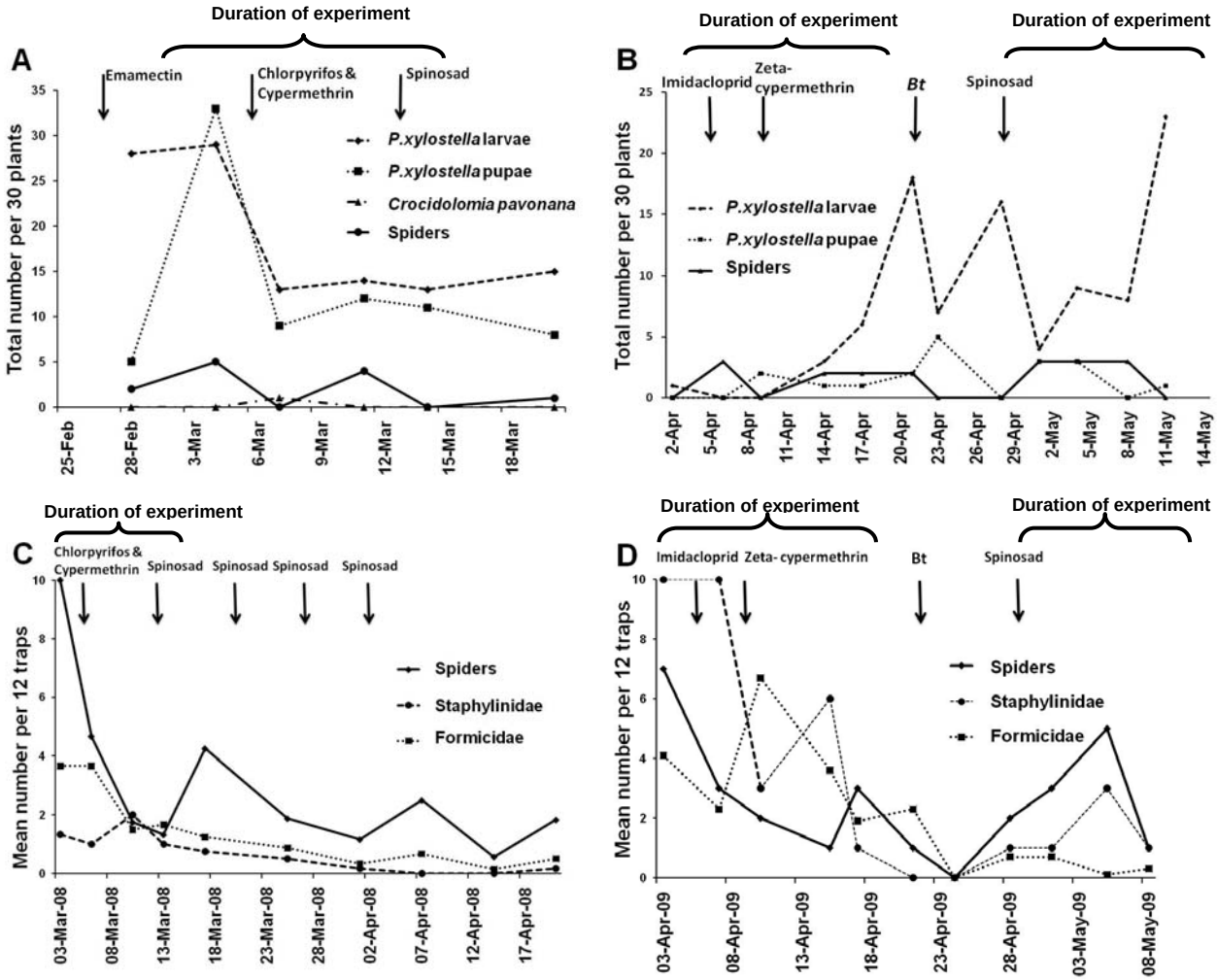

Figure 3. Major pests and arthropod predators sampled in cabbage crops. A) arthropods sampled on plants in the 2008 experiment; B) arthropods sampled on plants in the 2009 experiments; C) predatory arthropods sampled in pitfall traps in the 2008 experiment; 2008 experiment; D) predatory arthropods sampled in pitfall traps in the 2009 experiments. Arrows indicate insecticide applications.

Crop monitoring and insecticidal interventions

Plutella xylostella was the most prevalent pest attacking the head cabbage crop during the 2008 and 2009 studies (Figs. 3A and B); *C. pavonana* and *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) were also recorded in the crops but in consistently lower numbers than *P. xylostella*.

Foliar dwelling spiders, followed by Staphylinidae and adult ladybirds (*Menochilus* sp), were the most abundant natural enemies sampled on crop plants in both 2008 and 2009 (Fig. 3A and 3B). In 2008 the crop experienced a total of 15 applications of insecticide; two of these applications occurred during the experiment, each caused a marked decrease in the abundance foliar dwelling spiders (Figure 3A) and probably contributed to the gradual decline in numbers of all predators captured in pitfall traps (Fig. 3C).

In 2009 the crop received four applications of insecticide; two applications during the first experiment and a further two applications during the second experiment (Fig 3B). The abundance of foliar dwelling spiders decreased immediately after insecticide applications but then recovered (Fig. 3B). The abundance of epigeal predators declined following applications of imidacloprid and zeta- cypermethrin early in the crop but gradually increased as the crop matured despite applications of Bt and spinosad (Fig 3D); indicating that these insecticides are less harmful to epigeal arthropod predators.

However, quantitative comparisons between pitfall traps over the duration of the experiment, and hence inferences about the likely effects of different insecticides on ground dwelling predator populations should be interpreted with some caution. Pitfall traps typically trap large numbers of arthropods soon after they are set and subsequent catches tend to be lower (Schirmel *et al.* 2010).

Diadegma semiclausum is well established in the highland regions of Indonesia, including West Java (Sastrosiswojo and Sastrodihardjo 1986). In the current study the parasitoid made a significant contribution to *P. xylostella* population suppression in commercial cabbage crops despite frequent applications of insecticides, including broad spectrum pyrethroids. Such products have been shown to be highly disruptive to *D. semiclausum* populations in Australia, resulting in their localized exclusion from farms and subsequent increases in *P. xylostella* survival rates (Furlong *et al.* 2004ab). It is possible that the *D. semiclausum* population in the study area has developed a level of tolerance to some insecticides due to the high selection pressures to which they are exposed, or that immature parasitoids might be protected from insecticides while developing within insecticide-resistant hosts (Furlong and Wright 1993). Liu *et al.* (2003) have reported increased insecticide tolerance in *Cotesia vestalis* Haliday (Hymenoptera: Braconidae), which is another *P. xylostella* endo-larval parasitoid. However, there are no confirmed reports of increased insecticide tolerance in *D. semiclausum* which is, in general, more susceptible to insecticides than *C. vestalis* (Furlong *et al.* 1994). A more likely explanation for the efficacy of *D. semiclausum* in the study agro-ecosystem lies with local farming practices. In the region around Lembang most commercial cabbage crops are cultivated on small farms (< 0.5 ha) within a diverse multiple cropping system which consists of simultaneous crops of cauliflower, Chinese cabbage, tomato and kidney beans which are grown year round (Adiyoga, pers comm.)**.** The cropping intensity index in the region is typically between 250-300%, as farmers usually intercrop, thereby increasing crop heterogeneity further. This complex agro-ecosystem likely contributes to the persistence of *D. semiclausum,* as host plants for its host are always apparent and the patchwork of crops across the landscape is likely to provide spatial and temporal insecticide-free refugia. This cropping system is also likely to enhance the contribution of generalist predators to pest mortality. It has been demonstrated that arthropod predators frequently move between small crop plots (Prasifka *et al.* 2005) and the patchwork of crops in the study system is likely to provide shelter, a range of available prey items and refugia from insecticides that will facilitate predator persistence and thereby promote their contribution to pest suppression.

CONCLUSION

- Survivorship of *P. xylostella* and *C. pavonana* was significantly higher in the exclusion cages than in open cages, indicating that endemic natural enemies can have a significant impact on pest populations in this agro-ecosystem.
- The endemic natural enemy complex in the agro-ecosystem was able to reduce the net reproductive rate of *P. xylostella* <1, indicating that it has the capacity to cause pest populations to decline.
- Heavy rainfall events close to the time of *P. xylostella* egg hatch resulted in significant egg/ neonate mortality, demonstrating that abiotic mortality can also be significant and may serve to suppress pest populations.
- Despite the intensive use of a wide range of insecticides in the study agro-ecosystem, *D. semiclausum* was the major biotic mortality factor acting on *P. xylostella* populations in both 2008 and 2009. The mechanisms facilitating parasitoid persistence and its high efficacy in these conditions are unknown. However, the highly mixed, patchy landscape in which small areas of different-aged *Brassica* crops are continuously grown in close proximity to each other is likely to provide constant refugia for *D. semiclausum* and other natural enemies. It is also possible in this environment that *D. semiclausum* has evolved some degree of insecticide tolerance. Both possibilities deserve further research.

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Diurnal behavior of naturally microsporidia-infected *Plutella xylostella* and its major parasitoid, *Diadegma semiclausum*

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ABSTRACT
The diurnal behavior of the naturally microsporidia (*Nosema* sp)-infected diamondback moth (DBM), *Plutella xylostella* and its main parasitoid, *Diadegma semiclausum* (DS) was studied in the laboratory. Six behaviors were observed (from 0900 – 1100 hrs and from 1500 – 1700 hrs), namely flying, resting, moving, grooming, feeding and parasitism. There was a significant difference ($P < 0.05$) in the number of times each behavior occurred and the total time spent among behaviors by both DBM and DS both in the morning and afternoon. DBM and DS move, rest and parasitize (DS) significantly more frequently than fly, groom and eat. They also spent more time resting and moving around the experimental arena in both morning and afternoon sessions. A somewhat similar trend was also demonstrated by DS. The grooming and moving behaviors of DBM were positively and significantly correlated with the *Nosem*a spores concentration in its body during the morning session. In the afternoon, however, the resting, moving and flying were positively and significantly correlated with spore concentrations. Interestingly, in the morning session, except for parasitism, all DS behaviors observed were positively and significantly correlated with the spore concentrations in the body. In the afternoon session, relatively similar results were observed except for resting behavior. It was also found that DBM female adults laid significantly fewer eggs after five days of emergence than in the 1st to 4th days after emergence. In contrast, the numbers of eggs laid having *Nosema* spores significantly increased from day 1 to day 5 after emergence. Results of this study indicate that diurnal behavior of both DBM and its parasitoid were influenced by the amount of spores they carry; the most negatively affected is the parasitism behavior. Although each individual DBM adult laid ~~many~~ fewer eggs when severely infected by *Nosema*, the field population is expected not to be severely affected. In conclusion, results of this study revealed that the *Nosema* cannot be integrated with parasitoids such as *D. semiclausum* in DBM management programs.

Keywords
Microsporidia, *Plutella xylostella, Diadegma semiclausum,* Diurnal Behavior, Parasitoid, IPM

INTRODUCTION
The diamondback moth (DBM), *Plutella xylostella* L., is one of the major insect pests of cruciferous crops worldwide (Idris and Grafius 1995) and in Malaysia it has been recorded since 1925 (Ooi 1986). DBM is also one of the insect pests that are capable of developing resistance to all insecticides (including the microbial insecticides such as *Bacillus thuringiensis*, Bt) used against them (Talekar and Shelton 1993, Tabashnik et al. 1987, 1990). Efforts in integrating biological control agents such as predator and parasitoid with insecticides in managing the DBM is the best way to reduce the problem of resistance development as well as the cost of controlling this pest (Idris and Grafius 1995, Talekar and Shelton 1993). *Diadegma insulare* and *D. semiclausum* are the main parasitoids of DBM and were reported to be able to parasitize between 60 and 90% of the DBM population (Harcourt 1963, 1986, Idris 1995). In Malaysia, *D. semiclausum* has been the major parasitoid of DBM infesting cabbages planted in highlands such as the Cameron Highland. Interestingly, microsporidia such as *Nosema* spp. and *Vairimorpha* spp. are two main insect pathogens naturally infecting DBM populations both in high and lowland cabbage-growing areas (Batoto et al. 2010, Idris and Sajap 2003, Canning et al. 1999, Haque et al. 1999, Canning 1986). Although these pathogens are good candidates to be used with insecticides, parasitoids and predators in integrated management (IPM) of DBM, they are able to indirectly inflict a negative influence on the DS role in management of DBM in Malaysia (Idris et al. 2007, Idris and Norhayati 1997). *Nosema* for example, have caused a reduction in parasitism rate of DS on DBM as high as 60% in Malaysia (Idris et al. 2007). So, what is actually happening as the parasitoid gets indirectly infected with microsporidia? We hypothesize that *Nosema* infection to a certain extend may help in lowering the DBM field population but negatively affects the *D. semiclausum* role as an effective biological control agent of DBM. The objective of our work was to study the effect of microsporidia on diurnal behavior of DBM and *D. semiclausum.*

MATERIALS AND METHODS

DBM behavior

DBM larvae (2^{nd} –4^{th} instars) and pupae were collected from cabbage fields of Sg. Palas (1800 asl), Brinchang, Cameron Highland, Pahang, Malaysia, the famous highland cabbage-growing area in Malaysia. These different developmental stages of DBM were then reared until adult stage where fresh glasshouse-grown cabbage leaf was used to feed the larvae. A total of ten individual 2-day-old newly emerged DBM female adults were randomly selected from the stock and were put individually into 11 cm height x 12 cm diameter transparent plastic containers (experimental unit) with screen lids and sides. Cotton wool wetted with 10% honey was placed inside the container as food for DBM adults. The containers with the DBM were then placed in growth chambers with 20 $\pm$ 2°C temperature and 65% relative humidity and lighted by FL 36-6500 daylight. The containers were labeled 1 to 10 to help in recording the observed behavior (flying, feeding/eating, moving, grooming and resting) (Table 1) of each individual adult. The observation from the outside of the growth chamber was made from 0900 to 1100 h and 1500 to 1700 h. These times were selected based on reports of Ooi (1986). The numbers of each behavior and time spent for each behavior were recorded per 2-h observation.

Table 1. Definition of each behavior used in this study

Behavior	Definition
Flying	Opened wings, flew and landed inside the container observation arena and closed wings.
Resting	DBM adult did not move although the antennae were moving.
Moving	Involved any non-static movement except flying
Grooming	The insect did not move but used both hind legs to rub wings as well as using front legs to rub both antennae several times
Eating	DBM adult used its proboscis to suck up the honey from the diluted honey- wetted cotton wool.

Nosema spores in female adults

DBM adults used in the above observation were killed by squeezing its head after which the wings, legs and antennae were detached leaving the bare body. This is to avoid any *Nosema* spores on these body parts to influence the count of spores within the DBM body. The bared DBM body was put into 1.8 ml Wheaton tube filled with 0.25 ml distilled water and then crushed using a glass rod to create a homogenous spore suspension in distilled water. A total of 1.0 µl spore suspension was taken using 10 µl micropipette, which was then put on a hemacytometer slide (Bright Line Neubauer Model, American Optical Corporation Buffalo, New York, USA) and covered using slide cover. Spores were than observed and counted under the light microscope at 40x magnification. The number of spores were counted and estimated following the method of Cantwell (1970).

Data Analysis

Data on the number of each behavior and time spent among various types of behavior were analyzed using 1-way ANOVA. The relationship between each behavior and the abundance of *Nosema* spores in the DBM body was analyzed using regression analysis and run on the MINITAB Statistical program (Version 14).

DBM Parasitoid, *Diadegma semiclausum*

Diadegma semiclausum (DS) pupae were sampled from the same location as the DBM and put in transparent plastic containers as above but placed in the Coleman box provided with a cooler (blue ice) to ensure continuous development of the pupae without being affected by high temperature conditions on the way back to the laboratory. It was previously observed that DS cannot live long under temperatures of about 30°C, which is normal for Malaysia. The pupae were reared in the growth chamber in the laboratory at 23°C and 65% relative humidity. The emerged adults were sexed. The behavior of DS was studied as for DBM (Table 1) with an additional behavior of parasitism, which in this study was defined as the female DS touching the host larvae (DBM) using both of its antennae, followed by inserting its ovipositor into the host body, pulling out the ovipositor and leaving the host. Similarly, the abundance of *Nosema* spores inside the DS body was also studied as explained for DBM. Data were analyzed as above.

Number of eggs laid by field population of adult DBM and percentage of eggs with *Nosema* infection

DBM larvae and pupae were collected from the field as before and reared in laboratory at room temperature (25 – 30°C) and relative humidity (50%). Larvae were reared as above but were fed on artificial diet obtained from the Malaysian Research Development Institute (MARDI) until adults emerged. DBM adults (male and female) were mixed for two days after emergence. The females were then separated and put into transparent plastic containers as mentioned above, but each lid allowed insertion of aluminum foil egg oviposition sheets (Idris 1995). The Al foil was earlier wetted with the juice of *Nosema*-free cabbage leaf grown in the glasshouse of the National University of Malaysia (UKM) and air dried for 2 hours before being used. A total of 20 2-day-old DBM females was used in this study done in the laboratory environments as above. The numbers of DBM eggs were recorded daily (0900 h each day) until the 5^{th} day of treatment (7 days after emergence). The Al foil was

changed after the eggs were counted. Those counted eggs were subjected to spores observation using the same technique for DBM adults and DS. Data for the numbers of eggs laid and percentage of eggs with Nosema spores among the different days after treatment were analyzed by 1-way ANOVA and Tukey's test (P = 0.05) was used to separate the means when the analysis was significant.

RESULTS AND DISCUSSION

DBM Behavior

The mean total number of resting and moving behaviors showed by DBM was significantly (P < 0.05) more than other behaviors both in the morning and afternoon (Table 2). In the morning, however, DBM showed relatively more grooming behavior than in the afternoon, while flying and eating behaviors were seen more in the afternoon than in the morning. This indicates that in nature DBM adults are more active in the afternoon than in the morning (Idris and Grafius 1998). As such, if a pesticide is to be applied for controlling DBM, the afternoon would be better than in the morning.

Table 2. Mean total number (+ SE) of each DBM adult behavior in the morning (0900-1100 h) and afternoon (1500 – 1700 h)

Behavior	Morning	Afternoon
Flying	0.25 ± 0.12d	0.45 ± 0.14cd
Resting	3.02 ± 1.42a	3.25 ± 0.21a
Moving	2.21 ± 1.22ab	2.31 ± 0.84b
Grooming	0.72 ± 14.24c	0.25 ± 0.17d
Eating	0.21 ± 0.11d	0.63 ± 0.25c

Means in column with same letter are not significantly different (P > 0.05)

Table 3. Total time (sec) spent (+ SE) in the morning (0900-1100 h) and afternoon (1500 – 1700 h) by adult DBM

Behavior	Morning	Afternoon
Flying	1.62 ± 0.51d	0.33 ± 0.14d
Resting	5750.71 ± 631.42a	5598.01 ± 590.21b
Moving	1282.33 ± 580.22b	12,888.31 ± 581.84a
Grooming	124.72 ± 14.24c	1.56 ± 1.17d
Eating	42.51 ± 31.91cd	289.21 ± 50.25c

Means in column with same letter are not significantly different (P > 0.05)

DBM spent significantly (P < 0.05) more time resting and moving around the experimental arena and less time grooming, flying and eating (morning) and a somewhat similar trend in the afternoon (Table 3). Interestingly, moving and grooming behaviors (both morning and afternoon) were significantly correlated with *Nosema* spore concentration ($x10^{-3}$/µL) in DBM body (Table 4), indicating the disease affected certain DBM diurnal behavior. In view of this, and as suggested above, the DBM could be weakened by the disease infection and as such they would be more vulnerable to pesticide impact if application was in the late afternoon. After all, parasitoids of DBM, especially DS, are reported to be significantly less active in the afternoon than in the morning (Idris and Grafius 1998).

The mean total numbers of moving and parasitism behaviors showed by DS were significantly (P < 0.05) more than the other behaviors both in the morning and afternoon (Table 2). DS showed significantly more moving and parasitism behaviors than other behaviors in both sessions (Table 5), although these behaviors seemed to be less in the afternoon than in the morning. There was a significant (P < 0.05) difference in total time spent by DS among behaviors observed both in the morning and afternoon (Table 6). DS spent significantly more time moving than flying, resting, grooming, eating, or parasitizing. As for DBM, DS was expected to fly more but it turned out to be moving more than flying. This phenomenon would actually benefit us as far as DBM is concerned as DBM will become less productive (fewer eggs produced), which means less larvae feeding on cabbage leaves. In contrast, if DS spent less time for parasitism, it would negatively affect its role as an important biological control of DBM (Idris et al. 1997, 1998). A modification of parasitoid behavior by *Nosema pyrausta* was also reported by Orr et al. (1994).

Table 4. Relationship (r) between total time (sec) and *Nosema* spore concentration (x10-3/µL) in DBM body

Behavior	Morning	Afternoon
Flying	0.229 ns	0.425 ns
Resting	0.361 ns	0.595*
Moving	0.782*	0.761*
Grooming	0.595*	0.453*
Eating	0.275 ns	0.047 ns

ns = not significant; * = significant 'r' value

Table 5. Mean total number + SE) of each *Diadegma semiclausum* adult behavior in the morning (0900-1100 h) and afternoon (1500 – 1700 h)

Behavior	Morning	Afternoon
Flying	13.15 ± 3.41b	5.45 ± 2.10cd
Resting	9.02 ± 242bc	9.25 ± 4.11b
Moving	27.21 ± 6.24a	20.31 ± 5.84a
Grooming	6.22 ± 14.24c	6.85 ± 2.17bc
Eating	2.21 ± 0.81d	2.63 ± 0.75d
Parasitism	39.56 ± 5.13a	22.82 ± 2.52a

Means in column with same letter are not significantly different (P > 0.05)

Except for resting behavior in the afternoon, the time spent for each diurnal behavior including parasitism correlated with *Nosema* spore concentration ($x10^{-3}$/µL) present in their body (Table 7). This really shows that the disease is seriously affecting all diurnal behavior of the DS. Interestingly, the time spent for parasitism was negatively correlated with spore concentrations in each DS individual. This indicates that the parasitism rate of

DBM larvae in the field by DS will be negatively affected. This means that there is a need of other control measures to control DBM, and it is most probably insecticides. As the insecticide is used then the already highly insecticide-sensitive parasitoids like DS (Idris and Sajap 2003, Idris and Norhayati 1997, Geden et al. 1995, Idris and Grafius 1993a, 1993b and 1993c) will be more severely affected as they have been weakened by the disease. Similar impact is expected by other entomopathogens on DBM parasitoids (Furlong and Pell 1996, Kadir 1990). The negative impact of the *Nosema* infection was also reported on the insect predators, *Chrysoperla cornea* (Neuroptera:Chrysopidae) of European corn borer (Lepidoptera:Pyralidae) (Sajap and Lewis 1989).

Table 6. Total time (sec) spent in the morning (0900 – 1100 h) and afternoon (1500 – 1700 h) by adult *D. semiclausum* (DS)

Behavior	Morning	Afternoon
Flying	18.32 ± 0.51d	8.33 ± 3.64d
Resting	2061.7 ± 617.41a	1894.01 + 497.82b
Moving	48808.35 ± 580.13b	12888.13 + 581.82a
Grooming	148.73 ± 44.62c	1.55 ± 1.17d
Eating	126.52 + 103.48cd	289.23 ± 50.21c

Means in column with same letter are not significantly different (P > 0.05)

Table 7. Relationship (r) between total time (sec) and *Nosema* spore concentration (x10-3/µL) in DS body

Behavior	Morning	Afternoon
Flying	0.650*	0.719*
Resting	0.521*	0.344ns
Moving	0.964*	0.793*
Grooming	0.852*	0.555*
Eating	0.523*	0.558*
Parasitism	0.941 **	0.851**

ns=not significant; *=significant 'r' value; **=significant but negatively correlated

The mean number of eggs per field-collected female adult DBM was significantly (P < 0.05) different among days after emergence (Fig. 1). In contrast, as the DBM got older, the percentage of eggs with *Nosema* spores or the number of *Nosema*-infected eggs laid significantly increased. This phenomenon will definitely contribute to the low infestation level as there will be low egg hatching rate and fewer larvae successfully surviving to larger instars (3rd and 4th) (Batoto et al. 2010, Idris and Grafius 1999, Idris et al. 1997) that causes the major damage to the cabbage plants.

CONCLUSION

Nosema infection somewhat limits the diurnal behavior of DBM and most probably affects their reproduction rate (number of eggs laid). This will indirectly reduce DBM population in the field, and infected individuals may be susceptible to even less toxic pesticides. This is good news for farmers but the parasitism role of DS in controlling DBM ~~is~~ could be severely limited by disease infection. As such, the possibility of using *Nosema* or maybe other microsporidia species in an integrated pest management (IPM) program of DBM is questionable. Result of this study also indicated that any entomopathogen that seems to be effective in controlling a particular insect pest may not be useful in IPM as it could have negative effects on the role of parasitoids and predators of the pests.

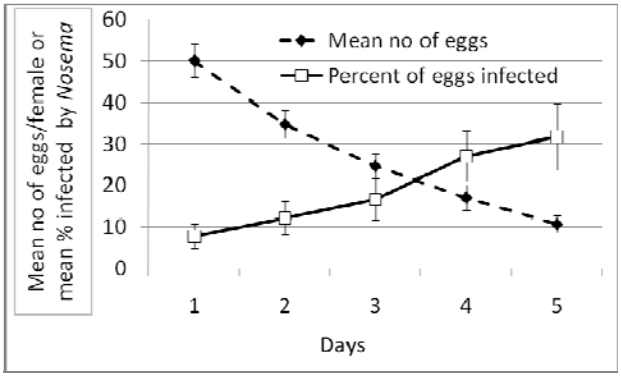

Figure 1. Mean (+ S.E) number of eggs laid (days after emergence) by *Nosema* –infected DBM female and mean percent (+ S.E) of eggs with *Nosema* spores

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Monitoring of diamondback moth in a cold-winter climate, South Island, New Zealand

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ABSTRACT
There has been a large increase in New Zealand's brassica production over the last few years, in particular kale (*Brassica oleracea* spp. *acephala*) and oil seed rape (canola, *B. napus* spp. *biennis*). Up to 300,000 ha are now grown annually, a significant proportion of which is grown in Canterbury, on the east coast of the South Island. Diamondback moth (DBM), *Plutella xylostella*, is one of the key insect pests on brassicas in New Zealand. Brassica growers in this cold-winter region are facing problems of insecticide resistance in DBM, and in particular, a lack of registered products with different modes of action for resistance management. An IPM program originally developed in the 1990s for vegetable brassicas grown in the North Island is being adapted and expanded into this region, where brassicas are often geographically separated, either due to lack of weedy reservoirs between crops or deliberate isolation of hybrid seed crops. In the 2008-09 year, a risk assessment survey using pheromone trap data showed that resident populations of DBM survived over winter in localized brassica crops. These emerged as moths in spring, re-infesting overwintering crops and later dispersing into spring sown crops. In the 2009-10 year, weekly pheromone trap catches of DBM were compared with corresponding larval infestations in five crops. Results show that increases in DBM larval infestations were detected 1–3 weeks (most between 2 and 3 weeks) after increases in pheromone trap catches. These results indicate the potential of DBM pheromone trap monitoring as a pest management tool for forecasting damaging larval infestations in a cold-winter climate.

Keywords
Integrated pest management, diamondback moth, pheromone trapping, crop monitoring

INTRODUCTION
Diamondback moth (DBM) *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is widely distributed around the world, and is the key pest of brassicas worldwide, including New Zealand (Talekar and Shelton 1993, Cameron et al. 1997). Extensive and regular use of insecticides against DBM on brassicas grown in New Zealand has led to detectable resistance to several insecticide groups, including synthetic pyrethroids and organophosphates (Bell and Fenemore 1990) and has been associated with control failures in three regions (Pukekohe, Gisborne and Hawke's Bay) (Cameron and Walker 1998, Walker et al. 2004a&b). In the 1990s, an integrated pest management (IPM) program for vegetable brassicas (focused in the North Island) was developed and implemented by Crop & Food Research (now Plant & Food Research) with support from the vegetable brassica industry, Horticulture New Zealand Limited (previously VEGFED) and the agrochemical industry. This program was initiated to counter the increasing levels of insecticide resistance in DBM (Berry et al. 2000, Walker et al. 2004a&b, 2009, 2011). The program is based on a cost-effective crop scouting system and uses action thresholds for cabbage, broccoli and cauliflower. It includes a DBM insecticide resistance management strategy that emphasises the rotation of products with selective activity and different modes of action to maximise the impact of existing biological control agents (Walker et al. 2009).

In the last few years, there has been a substantial increase in brassica production in Canterbury, South Island, New Zealand (171° 30' E, 37° 30' S, average elevation approx. 224 metres above sea levels). This increase is largely due to the expansion of forage brassica grown for dairy (predominantly kale, *B. oleracea* spp. *acephala*), growing of oil seed rape (canola, *Brassica napus* spp. *biennis*) for oil or biofuel production, as well as an increase in vegetable brassica production. As a result, there are now approximately 300,000 ha of brassicas grown annually in New Zealand (Walker 2009). Increased South Island brassica production has highlighted several issues that have the potential to impact on the success of the vegetable brassica IPM program, and in particular, resistance management of DBM. The main problems are 1) the key pest, DBM, is resistant to standard insecticides and there is a lack of registered products with different modes of action for use in resistance management strategies such as rotating insecticides over time, 2) important and effective natural enemies of some of the key pests are not present in parts of the South Island, for example *Cotesia rubecula* (Hymenoptera: Braconidae) introduced into some regions of New Zealand for the control of *Pieris rapae* (Cameron & Walker 2002), and *Asobara persimilis* (Hymenoptera: Braconidae) for the control of European leaf-miner,

Scaptomyza flava (GP Walker, unpublished data), and 3) brassica growers in sectors such as the dairy and seed industries are largely unaware of the IPM tools they can use to manage pests in their growing systems (Walker 2009). Therefore, a Ministry of Agriculture and Fisheries (MAF) Sustainable Farming Fund (SFF) project was initiated to improve foliage insect pest management in South Island brassica growing systems (vegetable, seed and forage), investigating the best way to control pests in this broader set of crops (Walker 2009, Anon. 2010). The project started in July 2008, initially focusing on defining the severity, timing and geographical distribution of the pest problems and the types of brassica cropping systems that were affected. The industry was concerned with where DBM populations were coming from (*i.e.*, overwintering or immigration), and developing a fast and effective method to monitor DBM to help predict damaging larval infestations.

One possible method for predicting larval infestations could involve pheromone trapping of male DBM moths. The efficacy of these traps has been proven in North Island vegetable brassica crops in New Zealand. However, pheromone traps have not been used by industry in the North Island mainly because these crops need regular monitoring for other spring and early summer pests (Walker et al. 2003). In South Island brassica production, this technology may prove more suitable because of the colder climate, isolation of some crops, lack of weedy brassica reservoirs and a smaller number of lepidopteran pest species. The aims of this study were to: 1) ascertain the origin of DBM infestations (*i.e.*, are populations establishing from dispersing individuals or from localized overwintering populations) using pheromone trapping and 2) evaluate the efficacy of predicting DBM larval populations in crops from pheromone traps capturing male moths.

MATERIALS AND METHODS

Pheromone Trapping

In the spring of 2008 (late September – early November), DBM pheromone traps were positioned in 16 brassica growing sites in Canterbury. Five sites were brassicas grown for vegetable seed production (2 autumn sown, 3 spring sown), five for forage brassica production (2 autumn sown, 3 spring sown), three for vegetable brassica production (spring planted) and three for forage brassica seed production (1 autumn sown, 2 spring sown). At each site, two traps were placed along the edge in the prevailing wind (in order to capture moths prior to their entering the crop), one at the northern end and one at the southern end of the crop edge.

Pheromone lures (Etec Crop Solutions (previously DeSire), Plant & Food Research (previously HortResearch)) were placed in the center of sticky bases in standard Delta® traps (28 cm length, 20 cm width, 13 cm height) (Etec Crop Solutions (previously DeSire), Plant & Food Research (previously HortResearch). Lures and sticky bases were replaced every four weeks. Sticky bases were replaced more regularly if they became covered with moths or dust.

Trap monitoring varied from weekly to monthly across the 16 sites. At all sites, traps were monitored until harvest stage or until numbers of moths in the traps indicated that DBM may be present throughout the area. Moths were counted cumulatively, so the total number of moths present on each trap was counted each time traps were checked, until the bases were replaced. The number of moths caught on each trap for each monitoring period (1 week – 1 month) was then calculated from these cumulative counts.

In the spring of 2009, DBM pheromone traps were positioned, as described above, in one newly planted crop at each of 11 brassica growing sites in Canterbury. Five sites were brassicas grown for vegetable seed production, four for forage brassica production and two for vegetable brassica production. Traps were monitored approximately weekly (range 5–9 days) until the crop was harvested or until numbers of moths in the traps indicated that DBM was present throughout the area. Traps were assessed in each crop as described above for the previous year.

Crop Monitoring

In addition to pheromone trapping in 2009, plants were examined for DBM larvae at all 11 brassica sites on the same days that the traps were inspected (approximately weekly). Plant monitoring continued until the crop was harvested or until the numbers of moths in the pheromone traps or numbers of larvae on the plants indicated that DBM were present throughout the area. At each site, the numbers of DBM larvae per plant were counted from 20 randomly selected plants along the prevailing wind edge of the crop (*i.e.*, the crop edge in which the pheromone traps were located). Twenty plants were sampled at each site until larval infestations increased to more than 2–3 plants with larvae per 20 plants, at which stage 30–40 plants were randomly sampled throughout the entire crop following the vegetable brassica monitoring technique developed by Beck et al. (1992).

Statistical Analysis

Pheromone Trapping

Owing to the scarcity of data collected in the 2008-09 year, a formal statistical analysis was not possible. Individual trap counts were converted to mean moth catch per day with the majority of data summarized in text, with data from two sites presented graphically.

For the 2009-10 year, data collected from five out of the eleven sites were statistically analyzed. Individual trap counts were converted to mean moth catch per day for statistical analysis. A generalized linear model with log link function and Poisson error distribution was used to estimate the seasonal pattern of DBM population density

in each crop. Standard errors of weekly means (SEM) have approximately constant length when drawn on a square root scale, so this scale is used on graphs, and the median SEM is shown. Data from the remaining six sites are not presented as nil to very few larvae were found on the plants, and/or the crop was severely damaged due to unforeseen circumstances such as extreme weather events.

Crop Monitoring

Groups of 10 consecutive plants were designated as replicates, and the total number of larvae found in each replicate was analyzed to reduce the number of zero observations to be modelled. A generalized linear model with log link function and Poisson error distribution was also used to estimate larval density per plant during the season. For each crop, data were included only from the date that larvae were first found on inspected plants.

Correlation between Pheromone Trap Counts and Crop Monitoring

The correlation over sampling dates of mean moth catch per trap per day and mean number of larvae per plant was calculated for each crop, for a range of weekly lags between moth and larval sampling dates from none to six weeks. Correlations between log(X+1) transformed means were also calculated.

RESULTS AND DISCUSSION

Year 1 (2008-09)

Pheromone Trapping

Placing pheromone traps in sites between late September and early November 2008 was considered early enough to catch any meaningful number of moths emerging from over-wintering sites. At the five autumn sown crops, moths were caught in every trapping period (one week – one month) in nearly every trap at every site from the time of trap placement. Initially (late September – early October) mean numbers of moths varied from 0.5–5.1/trap/day. Numbers then increased at all sites until the end of the trapping period (early December – early January), with mean captures ranging from 2.2 to as many as 10.6 moths/trap/day. For example, in a hybrid cabbage crop at Dorie, coastal Canterbury, 5.1 mean moths/trap/day were caught one week after traps were set up, with mean numbers then increasing to 10.6 moths/ trap/day by the end of the trapping period (Figure 1A).

These results show that DBM was able to survive the cold Canterbury winter, overwintering as resident populations in localized brassica crops (isolated seed and forage crops) sown in the previous season. Therefore, any brassica crops left over the winter season may harbour (resident) populations of DBM. Moths may then emerge in spring, re-infesting the same overwintering crops. Thus, in the 2008 season DBM infestations appeared to be highly localized early in the season.

DBM are known to adapt to relatively low temperatures for development (Liu et al. 2002) and survival (Honda et al. 1992, Saito et al. 1998). They have been found to develop successfully from egg to adult emergence at alternating temperature regimes ranging from 4 to 38°C. However, some life stages can develop significantly outside this range, especially at lower temperatures. For example, third and fourth instar larvae are able to survive temperatures lower than 4°C. In Hangzhou, China, DBM remain active throughout the year where the daily minimum temperature can frequently drop below -2°C (Ke and Fang 1979, Liu et al. 2000, Liu et al. 2002). Studies in southern and southeastern Australia have also shown that DBM populations can tolerate subzero temperatures, enabling them to survive over winter in brassica crops sown in the previous season (Ridland and Endersby 2006; Gu 2009). However, DBM does not survive in areas where the ground is frozen over winter, as brassica plants cannot survive and DBM must reinvade every year (Ke and Fang 1979; Liu et al. 2000; Liu et al. 2002). In Canterbury, the winter mean minimum daily temperature is 1.9°C and mean maximum daily temperature is 11.3°C (based on mean monthly values for the 1971–2000 period) (NIWA 2011). However, the minimum daily temperature frequently drops below 0°C. Therefore, if the ground is not frozen, it is possible for DBM to survive in all brassica crops grown over winter in Canterbury.

Conversely, at the 11 spring sown crops monitored in 2008, no moths were caught in the first week after traps were set up (late September – mid October) in any traps at all except at one site (0.2 moths/ trap/day). At these ten sites, low mean numbers of moths (ranging from 0.04–0.6/trap/day) were first recorded in traps 2–9 weeks after traps were set up. Numbers then increased at all sites until the end of the trapping period (early December – early January) with means ranging from 0.5–9.4 moths/trap/day. For example, in a red radish seed crop at Dorie, coastal Canterbury, no moths were counted in traps until eight weeks after traps were setup at which time 0.6 mean moths/trap/day were caught. Mean numbers of moths then increased until the end of the trapping period at which time 3.4 moths/trap/day were caught (Figure 1B). This was despite there being relatively high numbers of moths captured in traps located in a hybrid cabbage crop approximately 1 km south of this site (Figure 1A).

These results suggest that brassica crops planted in the spring may not be immediately infested with DBM, indicating that moths disperse into these new brassica crops later in the season. As our results show, DBM appear to be able to survive in brassica crops growing over winter in Canterbury, therefore it is likely that DBM infestations into ‘new’ spring sown brassica crops may come from local dispersal from neighboring overwintering crops within the region. Studies in southern and southeastern Australia also show that the bulk of DBM captured in spring sown brassica crops are derived from local populations dispersing from neighboring overwintering crops, rather than a series of

longer distance migrations into the area (Ridland and Endersby 2006; Gu 2009).

Year 2 (2009-10)

Pheromone Trapping and Crop Monitoring

Results from pheromone trap moth catches and crop monitoring for DBM larval infestations for five brassica crops are presented in Figures 2A–3C. Overall, moth catches were generally much higher in the 2009-10 season than in the previous season, with mean numbers of moths reaching peaks ranging from 14.9–32.9/trap /day in 2009-10 (cf. 0.5–10.6/trap/day in 2008-09).

As seen in the previous season, in all five spring sown brassica crops in 2009-10, zero to very few moths were caught in traps one week after traps were set up, with means ranging from 0–0.5/trap/day. Numbers of moths caught remained low at all sites in the first half of the season (early September/late November – mid December/early February) with means ranging from 0–2.9 moths/trap/day. Numbers of moths then increased at all sites over the second half of the season, peaking in early March at four out of the five sites, with mean captures ranging from 14.9 to as many as 32.9 moths/trap/day. For example, at Chertsey, numbers of moths caught remained low until late December (16 weeks after traps were setup in early September) with means ranging from 0–2.3 moths/trap/day. Numbers of moths caught then increased to 12.3/trap/day and then fluctuated between 3.7–14.8/trap/day before peaking at 23.9/trap/day in early March (Figure 2A).

When pheromone trap moth catches were examined in relation to larval densities for the five individual crops monitored in 2009-10, a three week lag produced the highest correlation for the two early spring sown crops while for the three late spring sown crops a two week lag produced a higher correlation. These results are illustrated in Figures 2A–3C, which show both mean moth catch per trap per day and associated mean larval count per plant over the season, with the larval means shifted back in time by the lags stated above. For example, at Chertsey an increase in moth numbers to 12.3/trap/day in late December occurred three weeks before an increase in larvae to 0.3/plant (Figure 2A).

Correlations between log(X+1) transformed data were used to assess the usefulness of trap catch to forecast larval populations. For all five brassica crops combined, the highest correlation ($r = 0.748$; $n = 61$) occurred for a lag of two weeks between moth and larval means. When larval data were restricted to sampling occasions after the first larvae had been observed in each crop, the highest correlation ($r = 0.705$, $n = 44$) again occurred for a two week lag, with one week and three week lags having the next highest correlations, with $r = 0.61$ and $r = 0.60$, respectively ($n = 44$).

These results show that an increase in DBM larval infestations within the monitored brassica crops occurred 1–3 weeks after increases in pheromone trap catches, with most occurring 2–3 weeks after these increases. These results indicate the potential of DBM pheromone trap monitoring as a pest management tool to help forecast risk periods when DBM larval numbers in crops are likely to increase to economically damaging levels. It is noteworthy that DBM populations were relatively low during this two-year study period, particularly in the 2008-09 year. This may have been partly due to factors affecting DBM in the Canterbury region in that year. A hot dry summer led to an outbreak of DBM in mid-summer (after pheromone trapping had finished) that caused considerable crop losses in the region, resulting in insecticides being applied to manage populations (GP Walker, personal observation). Immediately after this outbreak, DBM populations appeared to be decimated by an epizootic of the naturally occurring entomopathogenic fungus, *Zoophthora radicans* (GP Walker, personal observation). This event may well have contributed to the relatively low populations in 2009-10. However, despite the low populations of DBM, these results indicate that this technology may well reduce the time required by growers or crop managers to scout their crops and aid in decision making of when insecticides may be required for control of damaging infestations of DBM larvae.

CONCLUSION

Results from the 2008-09 year using pheromone trap data, showed that DBM could survive over winter as resident populations in localized brassica crops, emerging as moths in spring, re-infesting overwintering crops and later dispersing into spring sown crops. In the 2009-10 year, results from weekly pheromone trap catches of DBM, when compared with corresponding larval infestations in five crops, showed that DBM larval infestations increased 1–3 weeks after increases in pheromone trap catches, with most occurring 2–3 weeks after these increases. These results indicate the potential of DBM pheromone trap monitoring as an IPM tool for forecasting damaging larval infestations in a cold-winter climate where infestations appear to be highly localized early in the season.

This IPM tool could reduce the time spent scouting crops and aid in targeting any interventions, thus reducing the use of broad-spectrum insecticides and contributing to the sustainability of brassica production in the South Island of New Zealand.

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DairyNZ Ltd, Beef & Lamb New Zealand (previously Meat & Wool NZ), Bayer Crop Science, BASF New Zealand Ltd, Du Pont (NZ) Ltd, Dow Agrosciences (NZ) Ltd, Nufarm (NZ) Ltd, and Syngenta Crop Protection Ltd.

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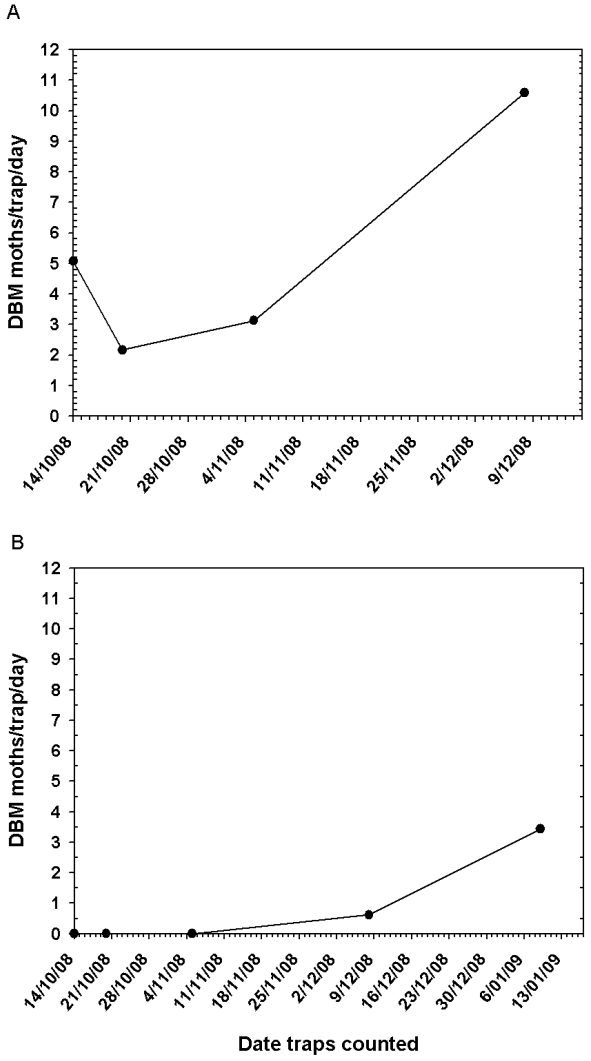
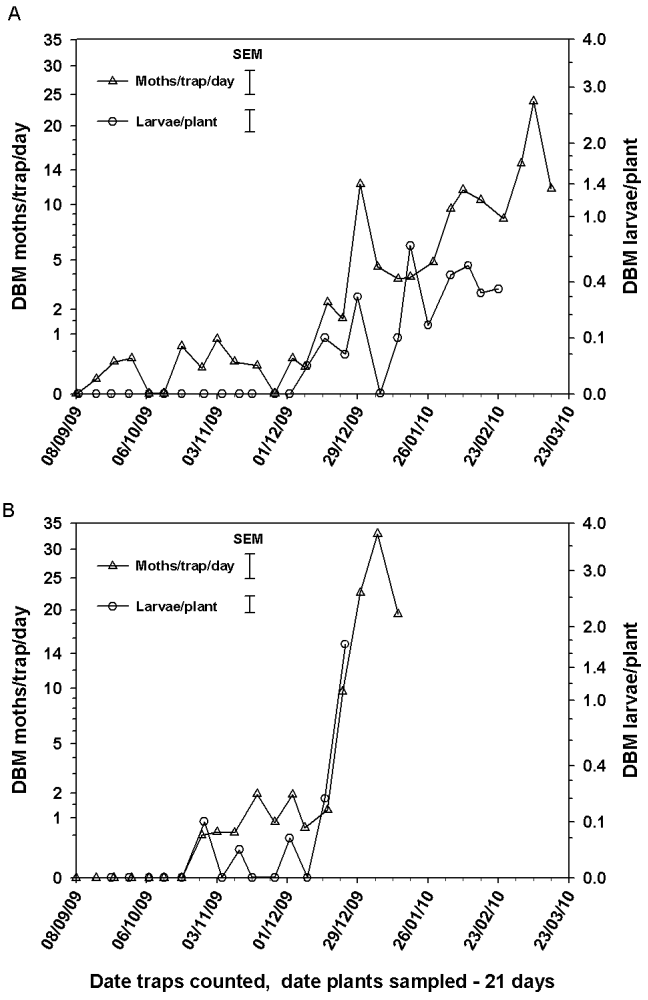
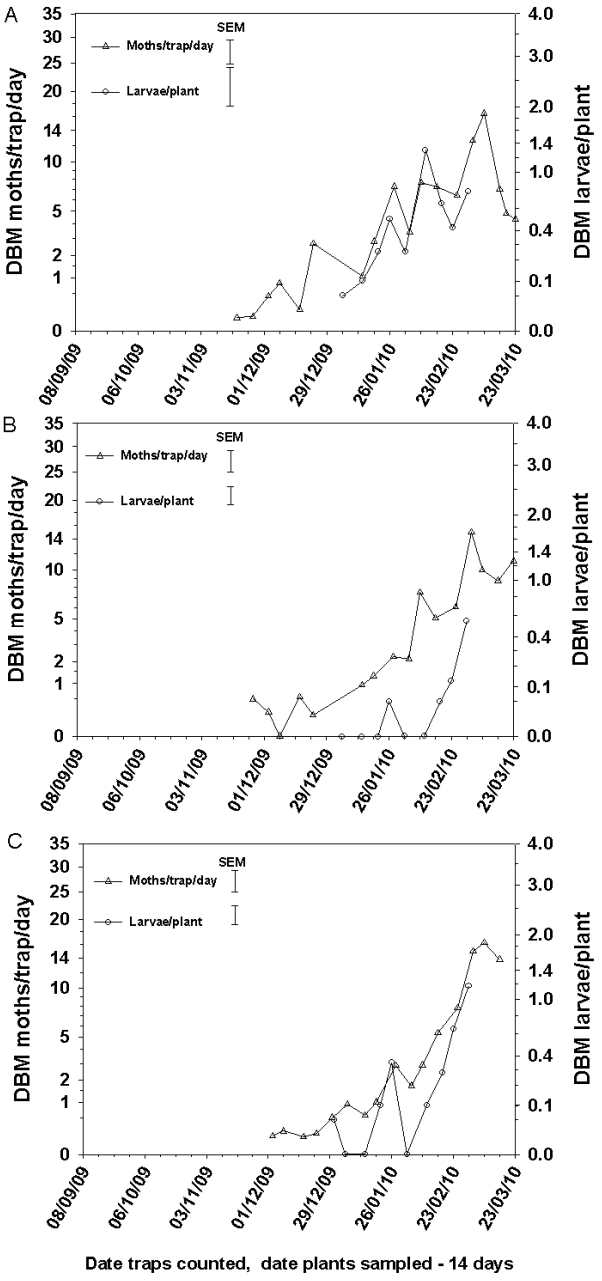
FIGURES



Figures 1A & B. Mean number of Diamondback moth (DBM) adults caught per pheromone trap per day in a hybrid cabbage seed crop (sown in autumn) and in a red radish seed crop (sown in spring) at Dorie, coastal Canterbury, in 2008-09



Figures 2A & B. Mean number of Diamondback moth (DBM) adults caught per pheromone trap per day and mean number of larvae per plant (plotted 21 days before scouting was carried out) in an early spring sown broccoli vegetable crop at Chertsey, mid Canterbury and in an early spring sown Chinese mustard seed crop at Dorie, coastal Canterbury, in 2009-10. Error bars show median SEM



Figures 3A, B & C. Mean number of Diamondback moth (DBM) adults caught per pheromone trap per day and the mean number of larvae per plant (plotted 14 days before scouting was carried out) in late spring sown kale forage crops at Rakaia, Dunsandel and Bankside, mid Canterbury, in 2009-10. Error bars show median SEM

Monitoring of cabbage looper, *Trichoplusia ni* (Hübner) populations inside and outside commercial greenhouses in western Canada

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ABSTRACT

Cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae), is a major pest of various field and greenhouse crops in western Canada. Each spring and early summer, adults migrate from southern California to as far north as Canada, but its overwintering success in western Canada is uncertain. In this study, cabbage looper populations were monitored inside and outside three commercial greenhouses in British Columbia over an entire year. Moth catches were highest in June, October and November in the pepper, tomato-1 and tomato-2 greenhouses respectively. During winter cleanup, no moths were caught inside the unheated greenhouses, but once the heating was re-established for the new growing season, adults were captured. No moths were caught in pheromone traps outside any greenhouse from December to March. These findings are important for understanding the temporal changes in infestations by cabbage loopers and their effective management.

Keywords

Cabbage looper, overwintering success, pheromone monitoring, greenhouse

INTRODUCTION

Cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae), is a generalist pest and has over 160 host plant species (Sutherland and Green 1984). It is of major concern in field and greenhouse vegetable crops in western Canada. Some greenhouse populations of cabbage looper have developed strong resistance in response to extensive *Bt* sprays in British Columbia, Canada (Janmaat and Myers 2003). Cabbage loopers are native to subtropical areas and adults are highly migratory. Each spring and early summer, adults migrate from southern California to as far north as Canada (Mitchell and Chalfant 1984). Recently, Cervantes et al. (2011) reported that cabbage looper pupae did not survive the winter outside in British Columbia but survived inside greenhouses possibly due to inadequate winter sanitations.

British Columbia produces ca. 2.8% of the volume of all Canadian field vegetable crops. A moderate climate and fertile land enable growers to produce a wide variety of field vegetables for fresh and processed markets. A diverse range of field vegetable crops are grown in British Columbia including potatoes, sweet corn, lettuce, cole crops, tomatoes, peppers and cucumbers (BC Ministry of Agriculture and Lands 2002); many of these crops are known hosts of cabbage looper. The production of greenhouse vegetable crops in British Columbia runs almost year round with the exception of a 2-6 weeks winter cleanup period. The production season begins in December/January and ends in November/December. Greenhouse crops are produced in soil-less growing media using hydroponic systems. The cleanup process takes place at the end of each production cycle. Winter cleanup generally involves removing the plant material, turning off the heating system, replacing growing media, applying cleanup insecticides, and washing irrigation infrastructure, walls and structures (Cervantes et al. 2011).

The aim of this study was to monitor cabbage looper populations inside and outside commercial greenhouses during spring, summer, fall and winter. By detecting the pest populations on time and delaying the introduction of moths to greenhouses, the continued need for insecticide applications could be reduced resulting in sustainable pest management.

MATERIALS AND METHODS

Monitoring experiments were conducted inside and outside three commercial greenhouses in Langley, British Columbia: pepper greenhouse (PGH), tomato greenhouse-1 (TGH-1), and tomato greenhouse-2 (TGH-2).

Monitoring of cabbage loopers inside greenhouses

Pheromone traps (Wing Trap II loaded with cabbage looper pheromone supplied by Contech Enterprises Inc., Victoria, British Columbia, Canada) were used to monitor cabbage looper populations inside the PGH, TGH-1 and TGH-2 from June 2002-May 2003. The monitored areas of PGH, TGH-1 and TGH-2 units were 15000 m^2, 7000 m^2 and 12000 m^2 respectively. Each greenhouse unit was monitored at one pheromone trap/1000 m^2. Pheromone lures were replaced every three

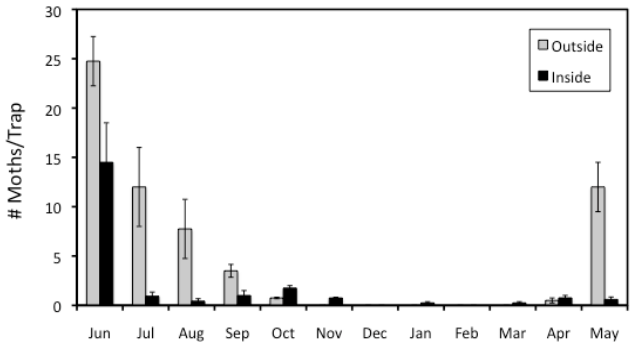

Figure 1. Pheromone trap catches (mean ± SE) outside and inside the pepper greenhouse from June 2002-May 2003 (data for winter months updated from Cervantes et al. 2011).

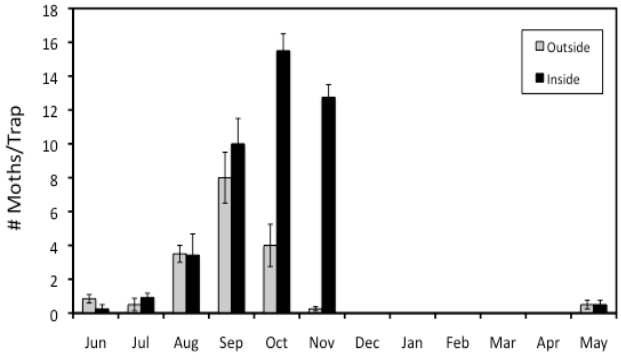

Figure 2. Pheromone trap catches (mean ± SE) outside and inside the tomato greenhouse-1 from June 2002-May 2003 (data for winter months updated from Cervantes et al. 2011).

weeks in spring and summer and every four weeks in fall and winter. Catches in traps were recorded weekly and the sticky trap inserts were replaced when more than three moths were caught.

Monitoring of cabbage loopers outside greenhouses

Pheromone traps were placed outside in the vicinity of three greenhouses at 2-3 traps per greenhouse side to cover wind flow from all cardinal points. Male captures were recorded at weekly intervals over an entire year (June 2002-May 2003) and the sticky trap inserts were replaced when more than three moths were caught. Lures were replaced every four weeks in fall and winter and every three weeks in spring and summer.

RESULTS AND DISCUSSION

Adult loopers were already established inside and outside the PGH, TGH-1 and TGH-2 when the pheromone trapping study started in June. Moth catches were highest in PGH in June whereas in TGH-1 and TGH-2 catches were highest in October and November respectively. Overall moth captures were higher in the TGH-2 than in the TGH-1 whereas captures in the PGH were the lowest (Figures 1, 2 and 3). During the unheated period no moths were caught in the greenhouses, but once the temperature was increased for the new growing season, adults were trapped inside the PGH and TGH-2 (Figures 1 and 3). Cabbage looper populations can persist year round inside greenhouses in British Columbia as pupae sometimes survive the winter cleanups (Cervantes et al. 2011). These greenhouse populations could therefore have 4-5 generations a year whereas field populations generally have only two generations.

No moths were captured outside any of the three greenhouses during winter but were caught in spring, summer and fall (Figures 1, 2 and 3), suggesting that loopers were not present in the monitored areas during winter. Langley is located north of 49° latitude and the mean minimum winter temperatures are generally well below 10 °C (Figure 4). Cabbage loopers are native to subtropical areas of North America, and are unable to survive over winter north of ca. 40° latitude when temperatures are ≤10 °C (Toba et al. 1973; Mitchell and Chalfant 1984). Previously, cabbage looper pupae placed outside were unable to survive winter conditions in British Columbia during two consecutive years (Cervantes et al. 2011). Evidence exists that some field

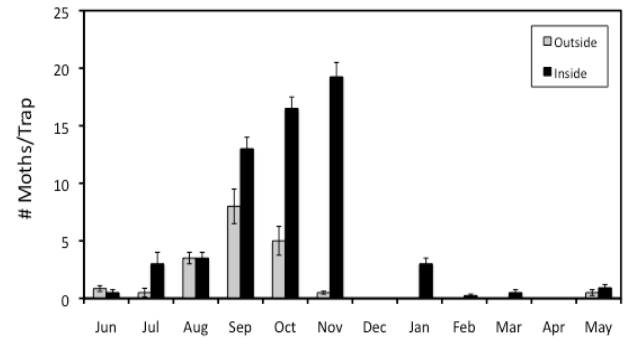

Figure 3. Pheromone trap catches (mean ± SE) outside and inside the tomato greenhouse-2 from June 2002-May 2003 (data for winter months updated from Cervantes et al. 2011).

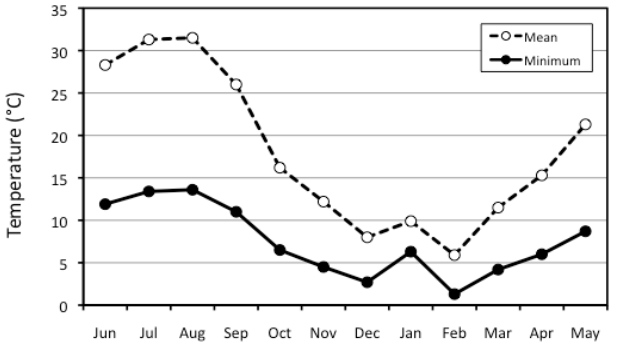

Figure 4. Mean and minimum temperatures from June 2002-May 2003 recorded at the Vancouver International Airport (data source: Environment Canada).

populations of British Columbia collected in spring were genetically similar to the California populations (Franklin et al. 2010), suggesting that early catches likely arrived from south. Moth catches outside tomato greenhouses were high late in the season, possibly associated with a greater flux of moths coming from inside the greenhouses when roof vents likely remain open.

Our findings suggest that cabbage looper populations fluctuate inside and outside greenhouses during spring, summer, fall and winter. Growers should ensure good quality winter cleanup to eliminate loopers inside greenhouses and maintain a window of low/no selection pressure as wide as possible to avoid continued resistance to insecticides. The lower the pest population densities at the end of the previous season the higher the chances to delay the first insecticide application the following season, and this can facilitate the reversion of the resistance acquired during the previous season.

Acknowledgements

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SESSION 3

Insect - plant interactions, chemical ecology and plant resistance

Importance of glucosinolates in determining diamondback moth preference and host range

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ABSTRACT
Glucosinolates are plant secondary metabolites used in host plant recognition by the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae). Among the plants on which *P. xylostella* oviposits, *Barbarea vulgaris* R. Br. (Brassicaceae) is highly preferred, despite its larvae not being able to survive on this plant because of its content of feeding-deterrent saponins. Recent research shows that the main glucosinolates, but not the feeding-deterrent saponins, are present on the leaf surface of *Barbarea* spp. plants in concentrations that can be detected by *P. xylostella* females. Experiments with sulphur fertilization also show that in *B. vulgaris*, sulphur fertilization can increase the plant content of the dominant glucosinolate and *P. xylostella* prefers to oviposit on the plants that have higher glucosinolate content as a result of sulphur fertilization. This paper focuses on *Barbarea* spp. as a model-system to study the effect of glucosinolates and other plant secondary metabolites on *P. xylostella*, and it also reviews how plant glucosinolate content affects *P. xylostella* in other plant species. Although host plant preference can be associated with a quantitative increase in glucosinolates, other aspects of the plant chemistry and morphology also seem to be involved in *P. xylostella* preference.

Keywords
Barbarea vulgaris, glucosinolates, oviposition, saponins, trap crop

INTRODUCTION
Glucosinolates are sulphur-containing plant secondary metabolites used for plant defense in crucifers (Halkier & Gershenzon, 2006; Hopkins *et al.*, 2009). In the case of herbivorous insects, glucosinolates generally play key roles in plant defense against generalists and are used in host plant recognition by crucifer specialists, such as the diamondback moth (DBM), *Plutella xylostella* L. (Lepidoptera: Plutellidae) (Reed *et al.*, 1989; Renwick *et al.*, 2006; Hopkins *et al.*, 2009). When presented individually or in mixtures, glucosinolates act as oviposition stimulants for DBM females (Reed *et al.*, 1989; Spencer, 1996; Sun *et al.*, 2009; Badenes-Perez *et al.*, 2010; Badenes-Perez *et al.*, 2011).

Whether glucosinolates are present on the surface of crucifer leaves where they can be detected by insect herbivores has been debated for years (Griffiths *et al.*, 2001; Reifenrath *et al.*, 2005; Hopkins *et al.*, 2009; Städler & Reifenrath, 2009). Recently, it has been found that glucosinolates do not appear to be present on the leaf surface of all crucifers, but they are present on the leaf surface of *Barbarea* spp. in concentrations that are detectable by DBM females (Badenes-Perez *et al.*, 2011). Before that, Griffiths et al. (2001) reported glucosinolates and DBM oviposition stimulation in the polar fraction of solvents in which leaves of several crucifers had been dipped, although glucosinolates could have originated from the inner plant tissue reached by the solvents through open leaf stomata. Studies with *Brassica napus* L., *Nasturtium officinale* R. Br., and *Arabidopsis thaliana* (L.) Heynh. did not detect glucosinolates on the leaf surface when searching for them with either gum arabic extractions or MALDI-TOF imaging (Reifenrath *et al.*, 2005; Shroff *et al.*, 2008).

Compared to other Brassicaceae, *Barbarea vulgaris* R. Br. is highly attractive to ovipositing DBM females, making it suitable as a trap crop (Idris & Grafius, 1996; Badenes-Perez *et al.*, 2004; Lu *et al.*, 2004; Shelton & Nault, 2004; Badenes-Perez *et al.*, 2005a; Badenes-Perez *et al.*, 2005b; Badenes-Perez *et al.*, 2006; Shelton & Badenes-Perez, 2006). Furthermore, larvae of DBM cannot survive on some varieties and types of *B. vulgaris* and on other *Barbarea* spp. because of their content of the saponins 3-*0*-*β*-cellobiosylhederagenin and 3-*0*-*β*-cellobiosyloleanolic acid, which act as feeding-deterrents or are correlated with deterrency (Shinoda *et al.*, 2002; Agerbirk *et al.*, 2003a; Badenes-Perez *et al.*, 2010; Badenes-Perez *et al.*, 2011). This paper focuses on *Barbarea* spp. to study the interaction between glucosinolates and saponins and DBM and it reviews the literature on the effect of plant glucosinolate content in DBM-plant interactions.

MATERIALS AND METHODS
Culture of insects and plants, analysis of glucosinolates and saponins, and bioassays were conducted as described in Badenes-Perez et al. (2011). Experiments with sulphur fertilization to increase glucosinolate content were conducted with *B.* vulgaris as in Badenes-Perez et al. (2010). Experiments to isolate and quantify

glucosinolates and saponins were conducted with *Barbarea rupicola* Moris, *Barbarea verna* (Mill.) Asch., *Barbarea vulgaris*, *Brassica napus*, and *Nasturtium officinale* plants (Badenes-Perez *et al.*, 2011).

RESULTS AND DISCUSSION

In our experiments with G-type *B. vulgaris*, sulphur fertilization increased glucobarbarin, the dominant glucosinolate of the plant, by 20% and DBM oviposited preferentially on the plants grown with sulphur fertilization (Badenes-Perez *et al.*, 2010). Similarly, *B. napus* plants grown in Hoagland solutions showed increased plant glucosinolate content as a result of added sulphur and the plants growing with sulphur were preferred by DBM compared to plants growing without sulphur (Marazzi *et al.*, 2004; Marazzi & Städler, 2004b). As sulphur-containing compounds, glucosinolates often increase when plants are fertilized with sulphur (Falk *et al.*, 2007). Sulphur fertilization could therefore make plants relatively more attractive to DBM, although in terms of trap cropping, it could make a trap crop for DBM more effective (Badenes-Perez *et al.*, 2010).

Glucosinolates have been detected in the surface waxes extracted from leaves of *B. rupicola, B. verna*, and *B. vulgaris* and there were also differences in glucosinolate content between abaxial and adaxial leaf surfaces within each plant (Badenes-Perez *et al.*, 2011). The concentrations of glucosinolates found on the leaf surface of *Barbarea* spp. were sufficient to be perceived by DBM females, but no glucosinolates were detected on the surface of *B. napus* and *N. officinale* (Reifenrath *et al.*, 2005; Badenes-Perez *et al.*, 2011), indicating that the occurrence of detectable levels of surface glucosinolates is species-specific. Further research is needed to test the presence of glucosinolates on the leaf surface of additional Brassicaceae and to check if the presence of surface glucosinolates could be associated with differences in ovipositional preference among host plants. The presence of glucosinolates on the leaf surface of *Barbarea* spp. supports their ecological role in host recognition and stimulation of oviposition for DBM (Badenes-Perez *et al.*, 2011).

Glucosinolates are not the only plant compounds active as oviposition stimulants for DBM and other insect specialists of Brassicaceae (Roessingh *et al.*, 1997; Marazzi & Städler, 2004a; Renwick *et al.*, 2006; Sarfraz *et al.*, 2006). Host plant selection by DBM females is influenced by chemical and physical stimuli (Badenes-Perez *et al.*, 2004; Sarfraz *et al.*, 2006). Waxes have been found to act synergistically in combination with glucosinolates, increasing DBM oviposition on substrates treated with sinigrin (Spencer, 1996; Spencer *et al.*, 1999). Glossy cultivars with low amounts of wax on the leaf surface are preferred by DBM over waxy cultivars despite lower survival of its larvae (Badenes-Perez *et al.*, 2004). Trichome density has also been shown to affect oviposition preference in DBM (Agerbirk *et al.*, 2003b; Handley *et al.*, 2005).

Larvae of DBM are able to desulphate glucosinolates and avoid their hydrolysis, which would result in isothiocyanates that can be toxic to DBM larvae (Li *et al.*, 2000; Ratzka *et al.*, 2002). When comparing the performance of DBM larvae on cultivars of the same plant species with different glucosinolate profiles, performance of DBM larvae could not be explained by plant glucosinolate content (Bodnaryk, 1997; Mosleh Arany *et al.*, 2008; Poelman *et al.*, 2008; Müller *et al.*, 2010; Sarosh *et al.*, 2010). However, one study found higher performance of larvae in plant lines with intermediate glucosinolate content (Siemens & Mitchell-Olds, 1996) and another study found higher performance of larvae in plant lines with low myrosinase content (Li *et al.*, 2000). Another study conducted in the field found higher densities of DBM larvae in plant lines with higher glucosinolate content (Bidart-Bouzat & Kliebenstein, 2008), although higher larval densities could be also due to oviposition preference for the plants with higher glucosinolates content.

Content of feeding-deterrent saponins 3-*0*-*β*-cellobiosylhederagenin and 3-*0*-*β*-cellobiosyloleanolic acid is associated with mortality of DBM larvae in *Barbarea* spp. (Shinoda *et al.*, 2002; Agerbirk *et al.*, 2003a; Badenes-Perez *et al.*, 2010; Badenes-Perez *et al.*, 2011). These saponins were not detected on the plant surface of *Barbarea* spp. despite their presence in the plant tissue, preventing DBM from being able to detect them (Badenes-Perez *et al.*, 2011). These saponins are also responsible for the resistance of *B. vulgaris* against the flea beetle *Phyllotreta nemorum* L. (Coleoptera: Chrysomelidae) (Kuzina *et al.*, 2009; Nielsen *et al.*, 2010). *Barbarea* is the only genus in Brassicaceae known to contain saponins, which might have originated later than glucosinolates, widely distributed in Brassicaceae (Nielsen *et al.*, 2010).

Further research with *A. thaliana* mutants, *Barbarea* spp., and a wide range of glucosinolate-containing plants in the Brassicaceae and other plant families containing glucosinolates is expected to clarify the importance of glucosinolates in determining host plant preference in DBM. Even within *B. vulgaris*, there are various chemotypes with different glucosinolate content and a total of 14 glucosinolates have been documented in this species (van Leur *et al.*, 2006; Agerbirk & Olsen, 2011).

CONCLUSION

The occurrence of glucosinolates on the leaf surface of *Barbarea* spp. clarifies the role of glucosinolates as token-stimuli in host recognition by DBM. Within the same plant type of *B. vulgaris*, the preference of DBM for plants with higher glucosinolate content as a result of sulphur fertilization can be used in trap cropping for DBM management. Further research with a wide range of glucosinolate-containing plants is expected to clarify

the importance of glucosinolates in determining host plant preference in DBM.

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Olfactory responses of *Plutella xylostella to* Chinese mustard volatiles

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ABSTRACT
Varieties of plants, when injured by herbivores, emit chemical signals that guide natural enemies to the herbivores. But, is the herbivore insect itself able to use these volatiles to get the information which it needs about its host? The objective of this study is to answer this question by testing the olfactory responses of diamondback moth (DBM), *Plutella xylostella* to Chinese mustard volatiles. Plants used in this study were damaged mechanically, by detached insect body parts (thorns on the hind legs of grasshoppers), by aphids and by larvae of DBM. Results of the Y-olfactometer test indicated that the DBM adults elicit different responses to odours released from Chinese mustard plants injured in different ways. When the insects were offered two plants damaged by aphids *(Lipaphis erysimi)* and also by their larvae in Y-olfactometer test, they were significantly ($P< 0.05$) more attracted to plants injured by aphids than those damaged by their larvae. However, the DBM did not give the same reaction to plants damaged mechanically or by detached insect body parts. This study shows that a plant will emit different volatile compounds depending on the way the plant was injured, and that DBM uses chemical information to locate the right host, most probably, for its offspring survival and reduce competition for food.

KEYWORDS
Plutella xylostella, Y-olfactometer, Chinese mustard, Volatile compounds.

INTRODUCTION
Plants produce an enormous variety of natural products with highly diverse structures. These natural products fulfil important functions in the interaction between plants and their biotic and abiotic environment. (Springob and Kutchan, 2009; Bruinsma et al., 2009). Plant semiochemicals are known to produce a wide range of behavioural responses in insects. These volatiles may have a negative or positive effect on an approaching insect (Renwick, 2001). Several plant species defend themselves indirectly from herbivores by producing herbivore-induced volatile compounds that attract the natural enemies of herbivores (Gols and Harvey, 2008; Yuan et al., 2009). For the Lepidoptera, host finding and acceptance is largely the responsibility of the female adult, which must select a site for oviposition that is optimal for the hatching larvae. Members of the family Cruciferae are attacked by a variety of insects including diamondback moth. In recent years, the diamondback moth, *Plutella xylostella*, has become the most destructive insect of cruciferous plants throughout the world (Talekar and Shelton, 1993; Bhalla and Dubey, 1986). This moth feeds only on members of the family Cruciferae, which includes cabbage, broccoli, cauliflower, collards, rapeseed, mustard and Chinese cabbage. These plants are from the most important vegetable crops grown in tropical climate regions in Asia.

A variety of induced compounds are released from herbivore-damaged plants (Gols and Harvey, 2008; Yuan et al., 2009). These plant volatiles may contain information on the identity of herbivores (Vet et al., 1991; Drukker et al., 2000; Reddy and Guerrero, 2004; Bruce et al., 2005; Ibrahim et al., 2005).

MATERIALS AND METHODS

Insects:

Diamondback Moth *(Plutella xylostella)*

DBM insects were collected from small farms in Danau Desa and at the Kampong Batu Muda in KL Malaysia. The insects were reared on cabbage leaves in small cages. The insect rearing was carried out in a controlled environment at 27±2°C, 60±5% R.H. with a photoperiod of 16:8 (L:D). The insects had been reared in the entomology laboratory in MARDI since February 2010. Around 50-60 new emerged males and females were allowed to mate for 2-3 days in 3-4 separate glass containers covered with mosquito-netting. During the mating period the insects were provided with a 20% honey solution and they were offered fresh mustard leaves for oviposition. The leaves with the eggs were transferred to small plastic cages (20x15x30) covered with mosquito-netting and given fresh leaves for 15-20 days. The emerged adults were transferred by using insect aspirator to the rearing cages.

Aphid *(Lipaphis erysimi (Kltb))*

Aphids were collected from Chinese mustard plants grown in glasshouses at MARDI, Serdang, Selangor, Malaysia. The insects were reared on Chinese mustard plants in small cages in an Entomology laboratory in MARDI.

Plants:

The plants used were sown in plastic pots, size 18 cm diameter filled with soil. The seeds were bought from Sing Heng Huat company, Taman Bukit Puchong Malaysia. All the plants were grown under glasshouse conditions in MARDI. The Chinese mustard plants used in the experiments were grown in separate insect-proof cages (2. 5 x 2 x 8 m). These plants were cultured directly in (12 cm) plastic pots until the 6-leaves stage when they were removed to the laboratory to be used in the experiments.

No-Choice Test (Y-olfactometer):

The Y-olfactometer was used to study the differences in the reaction of the DBM adult to plants which were injured by different ways**.** Mated 2-3 day-old DBM adult females were tested individually using a two-way olfactometer**.** The instrument had a Y shape and was made of transparent Plexiglas (2.5 cm ID; stem 13 cm, arms 10 cm; stem-arms angle 120°). The arms of the Y-olfactometer were connected to two glass containers (7000 ml) where the plants were placed (odour source). A pump connected to the olfactometer pushed filtered air into both containers through silicon tubes. All materials used in the test were thoroughly washed in soap and water, rinsed in 70% ethanol, and dried after every three runs. Each insect was allowed to respond for 7 min and was used only once. 15-16 plants were tested in all experiments, except plants damaged by larvae of DBM and by aphids where only 8 plants were tested. The plant control was changed every three runs. Five insects were tested for each plant. All experiments were carried out in daylight at 26±2 C° and around 60 % relative humidity.

Tests were done at two days for each experiment, 8 plants (runs) per day. Two days before the experiments, the plants were damaged (mechanically or by insects parts) or by 50 to 60 individual second and third-instar larvae of DBM or 100 to 150 aphids which were transferred to the leaves of each plant. The plants were injured again one day before the experiment.

Types of plant damage:

Four types of plant damage were used in the study. The plants were damaged mechanically, by thorns on the hind legs of grasshoppers, by aphids (100 – 150 aphids), and by larvae of DBM (50 – 60 second and third instars larvae). All plants were injured two days, one day and one hour before experiments commenced.

RESULTS AND DISCUSSION

In the present study it was observed that female DBM responded differently according to types of plant damage. Whenever the naive female was given the choice between odour sources, it was found significantly more attracted to plants damaged by aphids than to plants damaged by DBM larvae. That indicates that these plants were very attractive (Table1). In all other experiments DBM adults did not give the same reaction to plants damaged by aphids or by other methods when it was given healthy plants like a control. It is sure that the damaged plants emit chemical compounds but it seems that they are not the same.

Fig 1 There was no difference between plants damaged by DBM larvae for 3 days in attracting DBM adults over the control (undamaged plant). 41% of 80 insects were attracted to the damaged plant, 47.5% to the undamaged plants and 15% had no response.

For the plants infested with 160 aphids for 3 days, the same results were obtained. 41.33% of 80 females preferred plants infested with aphids over those uninfected (32%) and 26.6% had no response. (Figure 2).

Figure 3 and Figure 4 present, respectively, the results of experiments where plants were damaged mechanically and by detached insect body parts (thorns on the hind legs of grasshoppers).

Results suggest infestation may enhance the attractiveness of mustard plants. This attractiveness was not always significant. But in the comparative experiment, the female *P. xylostella* exhibited preference based on types of plant damage. Our results agree with those of Reddy and Guerrero (2004), who reported that the effects induced by host plants on herbivore insect behaviour appear to be part of insect strategies to gain access to new feeding and oviposition sites.

Table. 1: Percentage of DBM adults tested in no-choice test (Y-olfactometer) with plants damaged by aphids and by DBM

	Percentage of tested insects (n = 40)
Plants damaged by DBM larvae	**13.75 %**
Plants damaged by Aphids	**60 %**
No-response	**6.25 %**

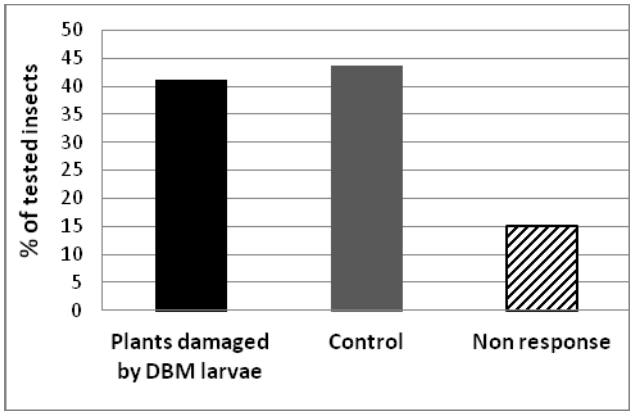

Figure. 1: Percentage of adult *P. xylostella* attracted to plants damaged by DBM larvae and to undamaged plants.

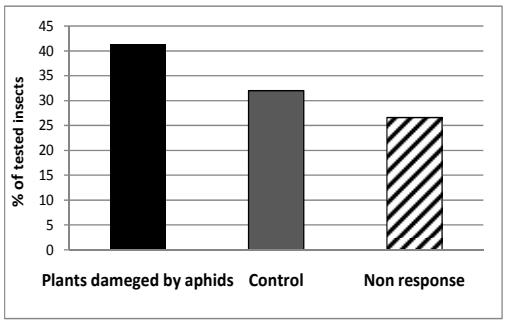

Figure. 2: Percentage of adult *P. xylostella* attracted to plants damaged by aphids and to undamaged plants.

CONCLUSION

In this study we have focused on the role of plant volatile compounds in providing information about the plant host and the capability of herbivorous insects to react to this information. We found that the adult DBM responded differently according to type of plant damage. The DBM female was found significantly more attracted to plants damaged by aphids than plants damaged by larvae of DBM.

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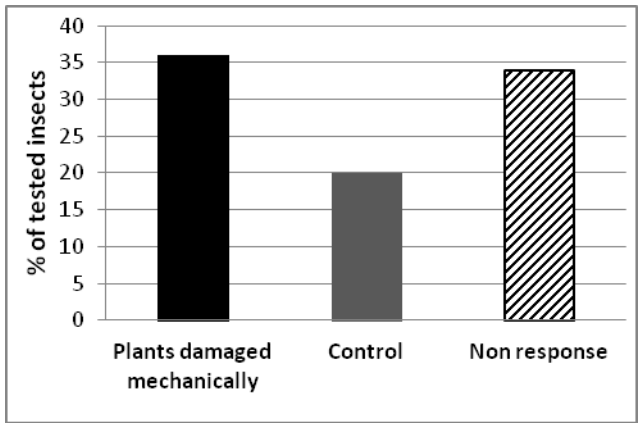

Figure. 3: Percentage of adult *P. xylostella* attracted to plants damaged mechanically and to undamaged plants.

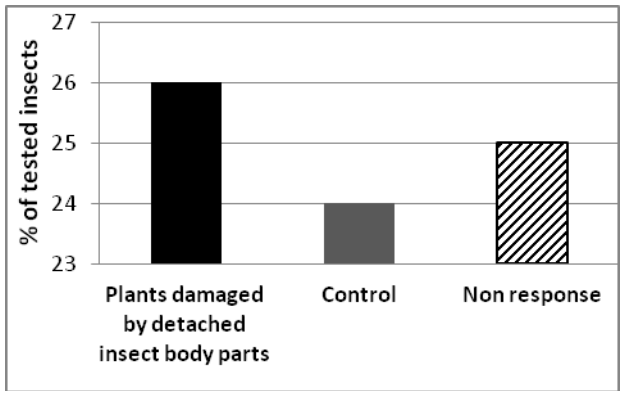

Figure. 4: Percentage of adult P. xylostella attracted to plants damaged by detached insect body parts and to undamaged plants.

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Host plant selection by *Crocidolomia pavonana* F. (Lepidoptera: Crambidae): effect of herbivory and adult experience

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ABSTRACT
When presented with a choice of *Brassica rapa* var. *chinensis* cv. Pak choy and *Brassica oleracea* var. *capitata* cv. KK-Cross in laboratory oviposition tests, female *C. pavonana* laid their eggs exclusively on *B. rapa*, indicating a significant preference for this host plant species. Similarly when gravid female *C. pavonana* were caged with single *B. oleracea* or *B. rapa* plants, the median time to oviposition for moths caged with *B. rapa* plants was significantly shorter than that for moths caged with *B. oleracea*. However, in olfactometer studies *C. pavonana* did not demonstrate a preference for the volatiles emitted by either *B. rapa* or *B. oleracea*, suggesting that visual cues and/or post-alighting cues may have an important role in the selection of host plants for oviposition. When gravid females were caged with single *B. oleracea* plants until they had accepted them for oviposition, they subsequently demonstrated a significant preference for *B. oleracea* over *B. rapa* in choice tests, indicating that oviposition preference is affected by adult female experience. Host plant selection was also affected by larval feeding damage, and *B. oleracea* plants which had previously been exposed to feeding by second instar *C. pavonana* larvae were significantly preferred for oviposition over undamaged *B. oleracea* plants. The diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae) co-occurs with *C. pavonana* as a pest of *Brassica* crops in tropical and sub-tropical regions of Africa, Asia and the Pacific and its choice of host plants for oviposition is also influenced by their damage status. The effects that co-occurring pests can have on the relative distributions of different species of insects in different crops is discussed, together with the implications that host plant choice affected by adult experience has for the employment of trap crops, as a management strategy for *C. pavonana*.

Keywords
Host plant preference; *Brassica*; adult experience; herbivory; trap cropping.

INTRODUCTION
Members of the genus *Brassica*, which is perhaps the most economically important of all genera within the Brassicaceae, are grown around the world as leafy vegetables or oilseeds (Gomez-Campo, 1980). However crop production is plagued by a plethora of arthropod pests. *Crocidolomia pavonana* F. (Lepidoptera: Crambidae), which is a native of Africa-Asia (Waterhouse and Norris, 1987), is a major constraint to *Brassica* crop production in tropical and sub-tropical regions of Africa, South Asia and across the Pacific (Waterhouse and Norris, 1987, Morallo-Rejesus and Navasero-Ward, 2003, Gunn, 1925, Sastrosiswojo and Setiawati, 1992). Although the host range of *C. pavonana* is generally considered to be restricted to members of the Brassicaceae, other plants such as Stickbrush (*Clerodendron fragrans peniflorum*) (*Verbenaceae*)) and *Tropaeolum* spp (*Tropaeolaceae*) have been reported as hosts (Gunn, 1925, Rai and Nagesha Chandra, 1977).

Crocidolomia pavonana usually occurs as a member of a complex of arthropod pest species that attacks *Brassica* crops in tropical and sub-tropical regions. Typically it is easily suppressed by applications of conventional broad spectrum insecticides which target other Lepidoptera within the complex. However, in situations in which the use of broad spectrum insecticides is eliminated, for example as part of IPM strategies which encourage parasitoids of the diamondback moth (*Plutella xylostella* L. (Lepidoptera: Plutellidae)), *C. pavonana* can increase in abundance. As it has only a small number of recorded natural enemies and few specialist parasitoids throughout much of its range (Waterhouse and Norris, 1987, Saucke et al., 2000), it often becomes the dominant pest species ((Morallo-Rejesus and Navasero-Ward, 2003) in the absence of broad spectrum insecticides. In tropical and subtropical regions the development of strategies to manage *C. pavonana* in a manner compatible with the conservation of diamondback moth parasitoids is a significant but increasingly important challenge for *Brassica* pest management.

Trap cropping is a traditional pest management practice which has the potential to make a significant contribution to IPM strategies (Hokkanen, 1991). In its simplest form, trap cropping can be defined as the use of a crop to attract and/or divert the arthropod pests away from the economically important crop to avoid damage, or concentrating them so that they can be economically destroyed (Silva-Krott et al., 1995). Trap cropping has been extensively studied as a tool for controlling diamondback moth in *Brassica* crops but results have been mixed (Badenes-Perez et al., 2004). The use of trap crops as a component of pest management strategies for *C. pavonana* management has received far less attention, but some studies (Silva-Krott et al., 1995, Srinivasan and

Moorthy, 1992) have reported that properly synchronized cultivation of Chinese cabbage (*B. rapa* var. *pekinensis*), Indian mustard (*B. juncea*) or radish in cabbage fields can divert *C. pavonana* away from the primary crop, thus reducing damage. However, host plant phenology can unpredictably influence the hierarchy of host plant preference of *C. pavonana* in a range of host plant choices (Smyth et al., 2003); this significantly complicates decision making underlying the choices of appropriate plants to be used as trap crops for *C. pavonana* management.

Almost all aspects of the ecology of *C. pavonana* are poorly understood. In this study we investigated some of the fundamental aspects of *C. pavonana* oviposition preference and behaviour. Interactions between adult and larval stages and different host plants were also studied in order to improve understanding of these basic relationships and to explore the possibility of developing trap cropping strategies as part of integrated crop management programs for *Brassica* vegetables.

MATERIALS AND METHODS

Plants

All plants used in experiments and insect cultures were grown in a greenhouse. Plants were grown in pots (12cm diameter) containing organic potting mix supplemented with fertilizer (Osmocote (5g NPK ratio, 16:3.5:10)); additional applications of fertilizer were made every 3 weeks. Two species of cabbage were used, Chinese cabbage (*Brassica rapa* var. *chinensis* cv. Pak Choy) and common cabbage (*Brassica oleracea* var. *capitata* cv. KK-Cross). To minimize the effect of host plant phenology on host plant preference by *C. pavonana* (see Smyth et al., 2003), Chinese cabbage plants were used 28- 42 days after transplanting and common cabbage plant were used 42- 56 days after transplanting; this ensured that plants of each species were comparable in leaf number, height and size.

Insects

A laboratory culture of *C. pavonana* was established from larvae and eggs collected from cabbage, broccoli and cauliflower fields in the Lockyer Valley in south-east Queensland, Australia. Larvae were fed daily on fresh excised leaves of *B. oleracea* in ventilated plastic containers (15 x 20 x 30 cm). When larvae reached the fourth instar, they were transferred into smaller ventilated plastic containers (15 x 10 x 8 cm) half filled with vermiculite as a pupation substrate. Adults emerged into these containers and were then transferred to oviposition cages (nylon mesh; 45 x 45 x 40cm) and supplied with 10% (w/v) honey solution as food source and potted plants *B. rapa* as an oviposition substrate. Eggs were collected from plants within 24 h of oviposition. All rearing was conducted in controlled environment cabinets (25 ± 2^{o}C; L: D 12:12h).

Oviposition studies

All oviposition tests utilized female *C. pavonana* which had been paired with male moths and held in plastic containers (15 x 10 x 8 cm). Moths were supplied with an adult food source (10% (w/v) honey solution) and incubated at 25 ± 2^{o}C; L: D 12:12h for 4 days to allow mating and the completion of pre-oviposition development. All oviposition tests were conducted using nylon mesh cages (45 x 45 x 40cm) at ambient temperatures and under natural light conditions in the laboratory. Adult moths were provided with an adult food source for the duration of all experiments.

No-choice tests

Oviposition on *B. oleracea* and *B. rapa* was studied by placing two plants of a given species into an oviposition cage together with an adult food source. A gravid 4-day old female moth and a male moth were then released into each cage; each experiment was replicated 30 times. Plants were examined daily and the number of egg masses, the number of eggs in each egg mass and the location of each egg mass were recorded. Observations were conducted until female moths died or for a maximum of 6 days.

Host plant preference tests

Oviposition preference for *B. oleracea* and *B. rapa* was tested using the standard oviposition method. Gravid 4-day old female moths were introduced into oviposition cages which contained a single *B. oleracea* plant and a single *B. rapa* plant; test plants were spaced 30cm apart within each cage. Plants were examined daily for the next three days and the number of female moths laying an egg mass on each species of plant was recorded. The experiment was replicated 30 times.

Effect of herbivory on oviposition on *B. oleracea* plants

Oviposition preference for *B. oleracea* plants damaged by larval feeding (20 second instar larvae foraging freely on the plant for 24 h) and undamaged *B. oleracea* plants was tested using the standard oviposition method. Gravid 4-day old female moths were introduced into oviposition cages which contained a single larval-damaged *B. oleracea* plant from which larvae had been removed and a single and undamaged *B. oleracea* plant; test plants were spaced 30cm apart within each cage. Plants were examined daily for the next three days and the number of female moths laying an egg mass on each plant was recorded. The experiment was replicated 10 times.

Effect of oviposition experience on host plant choice

Thirty gravid 4-day old female moths were paired with male moths and caged with single *B. oleracea* plants for a maximum of four days. Plants were examined daily and female moths removed from the cages once they had accepted the *B. oleracea* plant and deposited an egg mass. These moths were then introduced into an oviposition cage containing a *B. oleracea* plant and a *B. rapa* plant as previously described. Plants were examined daily for the next three days and the number of female

moths laying an egg mass on each species of plant was recorded.

Olfactory response of *Crocidolomia pavonana* to host plant volatiles

The response of *C. pavonana* female moths to the volatiles emitted by *B. oleracea* and *B. rapa* plants was tested in a Y-tube olfactometer (4cm diameter; 45 cm in length). Test plants were held in sealed glass chambers (18 x 18 x 18 cm) connected to each arm of the olfactometer, air was drawn through at a rate of 2l/ minute. Cohorts of 10 4-day old gravid female moths were introduced into the olfactometer at dusk in a darkened room; the number of moths in each chamber was recorded 12 h later. The experiment was replicated on 5 occasions over the period of one month. In a control experiment the response of *C. pavonana* female moths to the volatiles emitted by *B. oleracea* was investigated by comparing the response to these volatiles against the response to charcoal-filtered air.

Effect of larval diet on *Crocidolomia pavonana* survival and development

Five cohorts of 25 newly hatched *C. pavonana* larvae were reared on *B. rapa* or *B. oleracea* foliage in ventilated plastic containers (15 x 10 x 8 cm) held in environment controlled cabinets (25 ± 2°C; L:D 12:12h). Larvae were checked daily and supplied with freshly excised leaves of the appropriate food source as required. Larval survival and the duration spent in each instar were recorded and late fourth instar larvae were transferred to rearing containers half filled with vermiculite to act as a pupation substrate. Individual pupal weights were measured when pupae were two days old. Pupae were then transferred to individual Solo cups (35 ml) and reared to adults which were then sexed.

RESULTS AND DISCUSSION

No-choice tests

In no-choice tests, 93% of female *C. pavonana* accepted *B. rapa* for oviposition but only 66% accepted *B. oleracea*. *Crocidolomia pavonana* laid significantly more eggs masses on *B. rapa* plants (mean (±SE) = 5.3 (±0.7) egg masses per female) than on *B. oleracea* plants (2.9 (±0.6) egg masses per female) $F_{1,58}$= 8.12; *P*=0.006) (Fig. 1). However, oviposition patterns did not vary over the course of the experiment ($F_{5,348}$= 1.81; *P*=0.110) and there was no interaction between host plant species and time ($F_{5,348}$= 0.783; *P*=0.563) (Fig. 2A). *Crocidolomia pavonana* laid significantly more eggs on *B. rapa* plants (mean (±SE)= 191.6 (±19.1) eggs per female) than on *B. oleracea* plants (125.3 (±26.0) eggs per female) ($F_{1,347}$= 5.27; *P*=0.022) (Fig. 2B). Furthermore, egg deposition patterns varied over the course of the experiment ($F_{5,347}$= 2.93; *P*=0.013) and there was an interaction between host plant species and time ($F_{5,347}$= 2.75; *P*=0.019). Thus *C. pavonana* accepted *B. rapa* hosts more readily, depositing the majority of its eggs in the first few days of the experiment, whereas it deposited the majority of its eggs on *B. oleracea* much later in the experiment ((Fig 2B.)

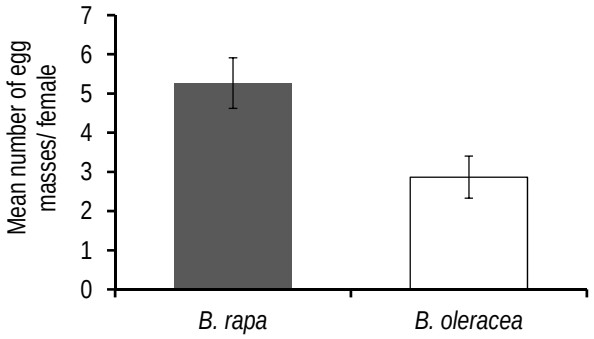

Figure 1. Mean number of egg masses laid per female (±SE) on each host plant species ($F_{1,58}$= 8.12; *P*=0.006)

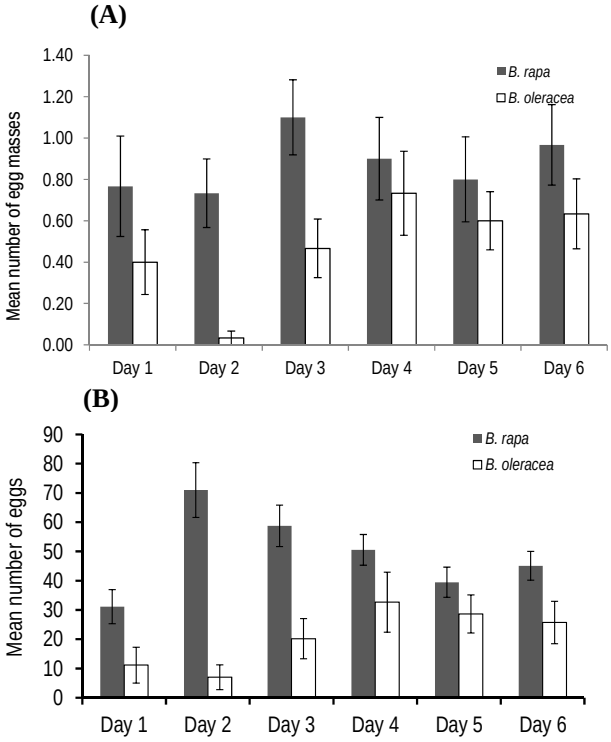

Figure 2. (A) Mean number of egg masses laid per female per day (±SE) and (B) Mean number of eggs laid per female per day (±SE)

Previous studies (Sastrosiswojo and Setiawati, 1992, Prijono and Hassan, 1992) showed that *C. pavonana* has a pre-oviposition period of 3-4 days during which eggs mature before the onset of egg laying. In this study, 4-day-old females were used to ensure females physiologically ready to oviposit when presented with a host plant. However, peaks in oviposition were observed 3-4 days after exposure to host plants (Fig. 2A), suggesting that the presence of a host plant may be an important determinant in the onset of egg development and maturation (Pittendrigh and Pivnick, 1993) and/or that host plant acceptance may be influenced by adult experience.

The egg masses laid on *B. oleracea* were significantly bigger than those laid on *B. rapa*. ($F_{1,244}$= 10.4;

P<0.0001) (Fig. 3). This apparent anomaly can probably be attributed to the delay in the acceptance of *B. oleracea* as a host plant for oviposition; as their egg loads accumulate females moths may became less discriminating, eventually accepting a less preferred host plant (Jallow and Zalucki, 1998) and then depositing large numbers of accumulated eggs as large egg masses. As *C. pavonana* laid egg masses which were highly variable in size, due to host plant effects and female egg load, egg mass number is probably a better measure of oviposition preference than the number of eggs laid, despite the later metric being used frequently in oviposition studies (e.g. Karungi et al., 2010).

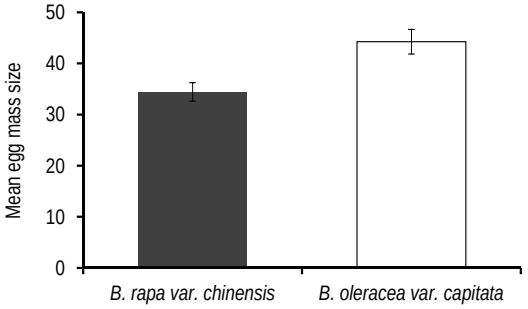

Figure 3. Mean egg mass size ($F_{1, 244}$= 10.4; *P*<0.0001).

Intra-plant distribution of egg masses

The intra-plant distribution of *C. pavonana* egg masses was similar between *B. rapa* and *B. oleracea* (Fig. 4). Egg masses were primarily deposited on the underside of lower and middle leaves but very few eggs were laid near the apical meristem, where early instar larvae subsequently feed (Takeuchi et al., 2009).

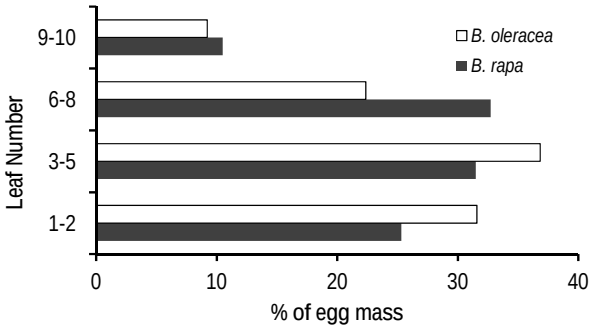

Figure 4.The intra-plant distribution of *C. pavonana* egg masses within *B. rapa* and *B. oleracea* plants (leaves numbered sequentially from the plant base).

Host plant preference tests

Of thirty gravid *C. pavonana* females subjected to an oviposition choice test between *B. rapa* and *B. oleracea* plants, all 25 moths which responded oviposited on *B. rapa*. This confirms previous reports that *C. pavonana* demonstrates a strong oviposition preference for *B. rapa* (Smyth et al., 2003, Karungi et al., 2010).

Effect of herbivory on oviposition on *B. oleracea* plants

When ovipositing females were given a choice of *B. oleracea* plants damaged by conspecifics and intact plants, females laid exclusively on damaged plants. This indicates that herbivore-induced changes make damaged plants more acceptable for oviposition. Similar results have been reported for *P. xylostella* oviposition on herbivore-damaged and intact *B. oleracea* plants (Lu et al., 2004), whereas herbivore attack of *B. rapa* plants makes them less preferred than intact conspecifics for oviposition by *P. xylostella* (Lu et al., 2004). Ongoing work is investigating the impact of herbivory of *B. rapa* plants on their suitability for *C. pavonana* oviposition.

Effect of oviposition experience on host plant choice

When female *C. pavonana* were restricted to *B. oleracea* for oviposition before being given a choice of *B. oleracea* and *B. rapa*, significantly more egg masses were laid on *B. oleracea* plants than on *B. rapa* plants ($F_{1,24}$= 7.55; *P*=0.011; Fig. 5).

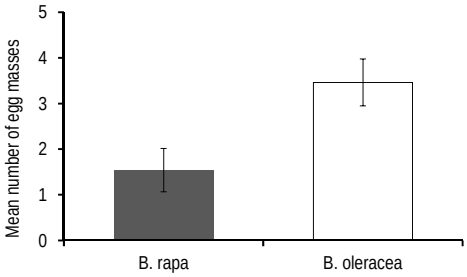

Figure 5. Oviposition preference of *B. oleracea*-experienced *C. pavonana*. Mean number of egg masses deposited on *B. oleracea* plants was significantly greater than the mean number of eggs masses deposited on *B. rapa* plants ($F_{1,24}$= 7.55; P=0.011).

This result is in direct opposition to the behaviour of naïve females which oviposited exclusively on *B. rapa;* it provides clear evidence that adult experience and learning can reverse innate oviposition preference in this species. This may explain the mixed successes reported in various studies on the use of trap crops to divert ovipositing *C. pavonana* away from the primary crop. The implications of the effects of oviposition experience on subsequent host plant choice for other *Brassica* pests are unclear. However, adult experience of neem volatiles has been shown to affect oviposition by *P. xylostella* (Liu et al., 2005) but the effect of host plant experience per se on subsequent oviposition by *P. xylostella* has not been studied in detail. Many studies have investigated the use of different trap cropping combinations against *P. xylostella* based on laboratory oviposition preference studies (Luther et al., 1996, Asman, 2002, Badenes-Perez et al., 2004, Silva-Krott et al., 1995) but only a small number of field studies have reported success (Srinivasan and Moorthy, 1992, Muniappan et al., 2004). Notably in

these studies the trap crop was planted at least 2 weeks before the main cabbage crop. In studies in which the trap crop was planted simultaneously with the main cabbage crop (Charleston and Kfir, 2000, Bender et al., 1999, Silva-Krott et al., 1995) the strategy was not successful in diverting oviposition from cabbage. The role of oviposition experience in such studies warrants further investigation.

Olfactory response of *Crocidolomia pavonana* to host plant volatiles

In olfactometer tests, *C. pavonana* females were attracted to volatiles from *B. oleracea* (Fig 6A) but not to filtered air. When presented with a choice of volatiles emitted from either *B. oleracea* or *B. rapa* plants in an olfactometer, naïve female moths did not show a preference (χ^2= 0.94; P>0.05; Fig 6B), despite ovipositing exclusively on *B. rapa* in dual choice tests. These results indicate that host plant volatiles are important in the pre-alighting and orientation of *C. pavonana* towards its host plants while post-alighting cues may play an important role in host plant acceptance. It is also important to note that these tests were conducted in the dark and that visual cues were absent; these could also be important in host selection by *C. pavonana*.

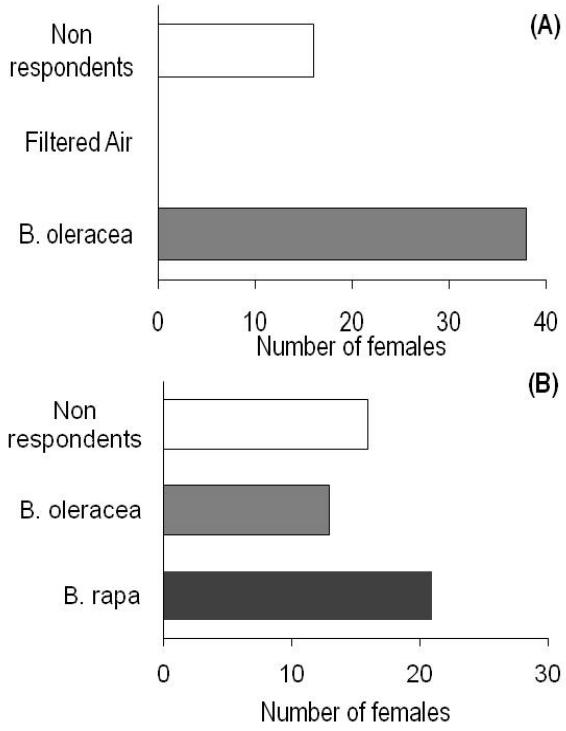

Figure 6. Olfactometer tests of the response of naïve female *C. pavonana* to volatiles from *B. rapa* and *B. oleracea*. (A) Response of moths to charcoal filtered air versus and *B. oleracea* volatiles. (B) Response of moths to *B. oleracea* volatiles versus *B. rapa* volatiles (χ^2= 0.94; *P*>0.05)

Effect of larval diet on *Crocidolomia pavonana* survival and development

Both *B. rapa* and *B. oleracea* were equally suitable for *C. pavonana* larval development and there was no difference in larval or pupal survival rates when insects were reared on the two species of test plant (Fig 7A). At 25°C, larval development from L1 to L4 was significantly shorter on *B. rapa* than on *B. oleracea* (Mann Whitney U- test z= 2.887; P=0.04; Fig. 7B) but pupae reared on *B. oleracea* were significantly bigger than those reared on *B. rapa* ($F_{3,128}$= 12.06; *P*<0.0.0001; Fig. 7C).

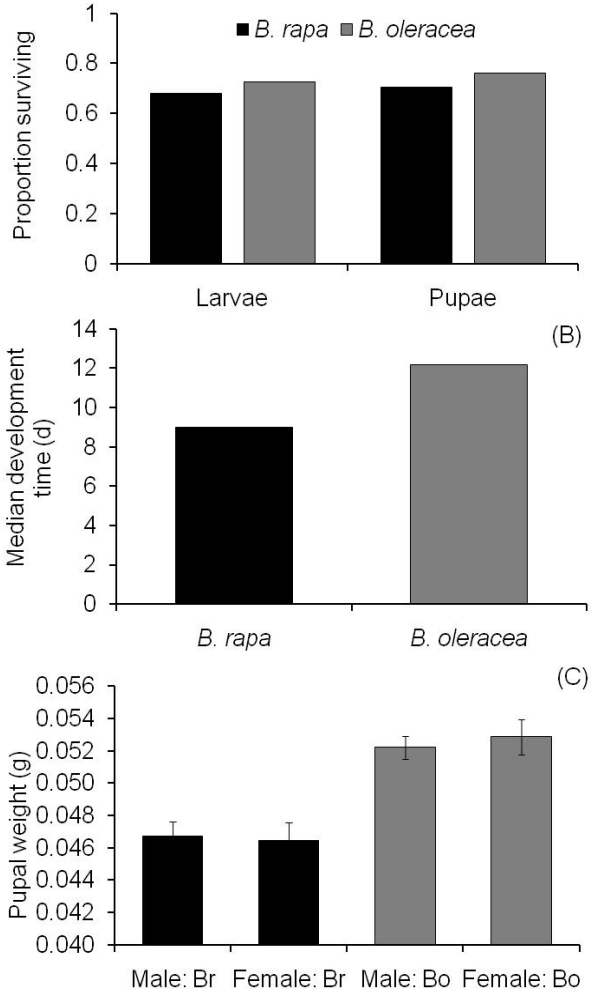

Figure 7: (A) Survival of *C. pavonana* larvae and pupae when reared on *B. oleracea* or *B. rapa*; (B) Larval development time (neonate to completion of fourth instar). Median development time was more rapid on *B. rapa* than on *B. oleracea* (Mann Whitney U- test z= 2.887; P=0.04); (C) Pupal weights of male and female moths reared on *B. rapa* or *B. oleracea*. Pupae of both sexes reared on *B. rapa* were smaller than pupae reared on *B. oleracea* ($F_{3,128}$= 12.06; *P*<0.0001)

CONCLUSIONS

- *Crocidolomia pavonana* expresses a clear preference for *B. rapa* over *B. oleracea* in oviposition tests. It accepts *B. rapa* plants more readily and lays more

egg masses and more eggs in total on *B. rapa*. In laboratory no-choice tests, egg masses laid on *B. oleracea* were larger than those laid on *B. rapa*; this is likely an effect of delayed host acceptance and reduced discrimination as egg load increases over time.

- Herbivore feeding increases the preference of *C. pavonana* for *B. oleracea*. Such induced physiological changes, together with changes in host plant preference with plant phenology, are likely to significantly affect *C. pavonana* oviposition patterns in the field, making them difficult to predict and complicating pest management strategies based on *C. pavonana* oviposition ecology and host-plant relationships.

- Following oviposition on *B. oleracea*, *C. pavonana* exhibits a clear preference for *B. oleracea* over *B. rapa* in choice tests, indicating that adult experience modulates host plant preference in this species. This has significant implications for trap cropping strategies. It suggests that trap crops should be planted well before the main crop, and raises important questions about the proximity of trap and main crops and impact that the origin of *C. pavonana* invading a crop will have on the effectiveness of a trap cropping strategy (this is likely to be determined by the *Brassica* crop mix at the landscape scale).

- *Crocidolomia pavonana* responds to host plant volatiles but naïve moths express no preference for the volatiles emitted by *B. rapa* (its preferred oviposition host) over the volatiles emitted by *B. oleracea*. This suggests that post-alighting cues might have a significant effect on oviposition, although the contribution that visual cues might make to the process still requires investigation.

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SESSION 4

Biological and non-chemical management of crucifer insects

Potential of entomopathogenic fungi and essential oils from aromatic plants in managing two lepidopterous cabbage pests in Indonesia

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ABSTRACT
The cabbagehead caterpillar, *Crocidolomia binotalis* (Zeller) and black cutworm, *Agrotis ipsilon* (Hufnagel), infest cabbage in Indonesia. These pests are traditionally controlled with synthetic insecticides which are expensive and hazardous to the environment. No effort has been made either to conserve the parasitoids/predators or to exploit them for the control of these pests. Entomopathogenic fungi and essential oils from aromatic plants can be used to develop alternative technologies to reduce these pests, and they are generally regarded as safe and more environment-friendly than synthetic chemicals. Besides, most of these materials are easily available and inexpensive. New isolates of the entomopathogenic fungi from field collections were assessed in the laboratory. There were significant differences in infectivity of all isolates tested against eggs and larval stages of this insect. The isolates of *Beauveria bassiana* (isolate BAP5 and BPP1), *Nomuraea rileyi* (isolate NAP3), *Metarhizium anisopliae* (isolate MAP 1), *Paecilomyces fumosoroseus* (isolate PPP4), and *Fusarium culmorum* (isolate FPP3) were more virulent to eggs and larval stages of *C. binotalis.* High levels of mortality were observed with *Beauveria* isolate at $1x10^7$ conidia ml^{-1}. The total percentage mortality of eggs and hatched larvae was 51.95%, and larval mortality of 50% was achieved in 1.71 – 6.94 days. Essential oils obtained from 17 aromatic plants of West Java Province, Indonesia, were screened for insecticidal activity against *A. ipsilon*. All of 17 essential oils *viz*., *Foeniculum vulgare, Lavandula angustifolia, Mentha* sp, *Cymbopogon nardus, Elsholtzia pubescens*, *Citrus grandis*, *Tagetes erecta, Cupressus sempervirens*, *Ocimum americanum*, *Cinnamomum verum*, *C. winterianus, Boesembergia pandurata*, *O. basilium*, *Pogostemon cablim*, *Ruta graviolens*, *C. citratus* and *Myristica fragrans* can affect *A. ipsilon* by direct or delayed insecticidal effect, through increased larval mortality with percentage of efficacy more than 70% and inhibition of *A. ipsilon* reproduction (both oviposition and adult emergence). *P. cablim, L. angustifolia, Mentha* sp, C. *verum*, *C. nardus, O. americanum* and *C. grandis* decreased pupal weight, pupation, adult emergence and egg hatchability. Four of the aromatic plants *viz*., *P. cablim, L. angustifolia, Mentha* sp and *C. verum* showed a feeding deterrent effect on *A. ipsilon* larvae at 10% concentration. These results suggest that naturally occurring entomopathogenic fungi and essential oils of aromatic plants could be useful for managing populations of *C. binotalis* and *A. ipsilon* on cabbage in Indonesia.

Keywords
Crocidolomia binotalis, *Agrotis ipsilon*, entomopathogenic fungi, essential oils, cabbage

INTRODUCTION
In Indonesia, cabbage is mostly grown in highlands. However, with the availability of cultivars which are also well adapted to lowlands, cabbage area in this agro-ecocystem is continuously increasing. In 2009, it was cultivated on a total area of 67,793 ha producing 1.36 m.t with an average yield of 20,03 t/ha (Statistics Indonesia 2009). A wide range of insects attack cabbage and they form the major limiting factor in its successful cultivation and yield improvement. Among them Cabbage head caterpillar (CHC), *Crocidolomia binotalis* (Zell.) (Lepidoptera: Pyralidae) (Sastrosiswojo and Setiawati 1983) and the black cutworm (BCW), *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) are the most destructive insect pests. BCW attacks the crop from the nursery stage onwards, and caused 75% loss in seedlings (Sastrodihardjo 1982).

These pests are traditionally controlled with synthetic insecticides which are expensive and environmentally hazardous. Farmers spray synthetic insecticides 2-3 times/week and the total number of pesticide applications

on cabbage cultivation in one season could reach 30-35 times (Rauf *et al*., 2005). Moreover, about 70% of cabbage farmers spent money for pesticides about 25-30% of the total production cost. Until now, only two insecticides have been recommended to control BCW in Indonesia. No effort has been made either to conserve the parasitoids/predators or to exploit them for the control of these pests.

The development of new techniques other than chemical insecticide was necessary to control these insects. Entomopathogenic fungi and essential oils from aromatic plants can be used to develop alternative technologies to reduce these pests, and they are generally regarded as safe and more environment-friendly than synthetic chemicals.

The role of fungal pathogens as natural enemies for CHC has recently been explored and several isolates of hyphomycetous fungi have been identified. *Fusarium* sp., *Beauveria* sp., *Metarhizium* sp., *Nomuraea* sp., *Paecilomyces* sp., and *Achersonia* sp. were found infecting CHC larvae. The fungus was multiplied on Sabauraud Maltose Agar + Yeast medium under laboratory conditions, and can also be mass produced in rice and wheat (Nuraida and Hasyim 2009). Hashim and Ibrahim (2003) reported that *Paecilomyces fumosoroseus* (Wise) Brown and Smith, *Beauveria bassiana* (Bals.) and *Metarhizium anisopliae* var. *majus* have been shown to be infective against the CHC. Tang and Hou (1988) reported that the entomopathogenic fungus, *Nomuraea rileyi* caused 90.5–100% mortality in fourth-instar larvae of the corn earworm, *Helicoverpa armigera*, when applied at 10^7 conidia/ml to corn silks, and leaves of soybean, tomato and chrysanthemum. The LT_{50} was 5.9–6.7 days.

Many plant essential oils show a broad spectrum of activity against pest insects ranging from insecticidal, antifeedant, repellent, oviposition deterrent, growth regulatory and antivector activities. The essential oil of plants is used as an alternative to pesticides for the control of insect pests. However, research provides limited information on the effectiveness of essential oils against *A. ipsilon*. Shadia *et al*. (2007) reported that the essential oils of *O. americanum* had larvicidal activity against *A. ipsilon*. All female moths resulting from larvae fed on leaves treated with basil for 24 h were deformed and all died before oviposition (Abd El-Aziz et al. 1997).

This study was conducted to assess the potential of entomopathogenic fungi and essential oils from aromatic plants for managing *C. binotalis* and *A. ipsilon* on cabbage in order to reduce the use of insecticides and to improve the quality of the product.

MATERIALS AND METHODS

Effect of entomopathogenic fungi against *C. binotalis*

Insect culture

C. binotalis larvae were collected from cabbage in West Sumatra. Larvae were fed with fresh cabbage and maintained in laboratory under controlled environment. Distilled water and 10% honey were provided separately as food for the adults. To obtain eggs and larvae of uniform age, plants with one day old eggs from oviposition cages were transffered daily into new cages and the eggs were allowed to hatch.

Fungal isolation and preparation

The fungal isolates were collected from soils using insect bait method (Goetel and Inglish 1977; Zimmermenn 1986). All the isolates were grown on Sabouraud dextrose Agar with yeast extract (SDAY) (dextrose 10 g, peptone 2.5 g, yeast extract 2.5 g, agar 20 g, in 1 L H_2O) (Samuels *et al.*, 2002). The inoculated SDYA with fungal spore was incubated for 7 days at 23–25°C (Rice and Cogburn 1999).

Eggs treatment

Two egg masses for each isolate were dipped in fungal spore suspension at the rate of 10^8 conidia mL^{-1} for 60 sec, and placed in a petri dish. Mortality of eggs was observed every day. The egg bioassay was repeated three times.

Larva treatment

The first, second, third and fourth instar larvae of *C. binotalis* were used in this experiment. Concentration of conidia of each fungus used was 10^7 conidia mL^{-1} . Fungi were inoculated by spraying conidial suspension (0.2 cc/20 larvae) in the dorsal and ventral part of larval body using a handsprayer. Larvae that had been sprayed were fed with fresh cabbage. The treatments were repeated four times and each treatment unit consisted of 20 larvae. Mortality of larvae was observed every day until seven days after the application of fungi.

Effect of essential oils against *A. ipsilon*

Insect culture

A. ipsilon larvae were collected from cabbage in Lembang District, West Java, Indonesia. Larvae were reared in the laboratory at the Department of Entomology of Indonesian Vegetable Research Institute (IVEGRI). Emerging adult moths were transferred to cages and fed on a 10% sucrose solution. Moths were transferred at ratio 1:1 for oviposition. The cage was covered with paper towel for egg laying. The paper towel containing eggs was removed daily. The paper towel eggs were moistened and kept in plastic containers to allow hatching. One day-old adults were used for oviposition deterrent activity. All experiments and mass rearing were carried out at ambient temperature (approximately 27 ± 2^0C).

Plant extraction

Essential oils were extracted from the leaves, fruits, entire plant, fruit and seeds of selected aromatic plant species (Table 1) by steam distillation.

Tabel 1 . Aromatic plants and the part of plants used

Plant source	Family	Plant part used
Foeniculum vulgare	Apiaceae	Fruit
Lavandula angustifolia	Lamiaceae	Flowers
Mentha sp	Lamiaceae	Leaves
Cymbopogon nardus	Gramineae	Leaves
Elsholtzia pubescens	Labiatae	Leaves
Citrus grandis	Rutaceae	Peels
Tagetes erecta	Asteraceae	Whole plant
Cupressus sempervirens	Cupressaceae	Leaves
Ocimum americanum	Labiatae	Seed
Cinnamomum verum	Lauraceae	Wood
C. winterianus	Gramineae	Leaves
Boesembergia pandurata	Zingiberaceae	Rhizome
O. basilium	Labiatae	Leaves
Pogostemon cablim	Lauraceae	Leaves
Ruta graveolens	Rutaceae	Leaves
C. citratus	Gramineae	Leaves
Myristica fragrans	Myristicaceae	Fruit

Antifeedant activity

The antifeedant activity of the essential oils were experimented against the third instar larvae of *A. ipsilon* using a choice test as described by Abd. El Aziz *et al*. (1997). Ten replicates were used for each test. The amount of food consumed per leaf disc was estimated using the following formula :

Deterrence (%) : (1-T/C) x 100

Where, T and C represent the percent leaf area (cm^2) consumed per larva of the treated and control sets, respectively

Insecticidal properties of tested plant extracts

The insecticidal properties of essential oils were tested on the third instar larvae of *A. ipsilon* as described by Abd El Aziz *et al.* (1997). The percentages of larval mortality and pupal mortality, pupal duration and pupal weight were recorded.

Ovipositional Inhibition and Repellency Tests

Cabbage var. Green coronet were planted in pots (20 cm dia). Five pots per essential oil were sprayed at a concentration of 1.0% using a hand sprayer (1 l capacity). Another five pots were sprayed with water and emulsifier as untreated check. The treated plants were placed in a wooden cage (100 X 100 X 100 cm), covered with screen. Thirty pairs of newly emerged moths were introduced in each cage. Each test was replicated three times. Eggs laid on the potted plants inside the cage were collected daily. The number of eggs deposited on treated or untreated plants was counted and the percent repellency values were calculated according to the equation D = (1- T/C) X 100, where T and C represent the mean number of deposited eggs per female in the treated and check set, respectively.

Ovicidal tests

Twenty eggs of *A.ipsilon* per treatment were exposed to the essential oils and allowed to hatch. The experiment was carried out with 18 treatments in five replications using Completely Randomized Design (CRD).

Statistical analysis

In all experiments, data was analyzed using Statistical Analysis System (SAS) 6,12 version. The comparison of means were done using Duncan's multiple range test at 0.05 level.

RESULTS AND DISCUSSION

Effect of entomopathogenic fungi against *C. binotalis*

Mortality of *C. binotalis* eggs as a result of exposure to different isolates of entomopathogenic fungi is presented in Table 2. The characteristic symptoms of eggs was loss of turgor, dullness and darkening of the eggs, and subsequently the eggs were dried. The *B. bassiana* (BAP5) isolate caused the highest mortality (51.95%). NAP3, BPP1 and FAP5 caused the second highest mortality of 28.42%, 26.90% and 23.36%, respectively, whereas the remaining isolates resulted in less than 20% mortality. The egg mortality obtained in the present study was higher than that observed by other authors such as Sahagun *et al.* (2005) who found that treatments with the fungi *M. anisopliae,* and *P. fumosoroseus* only reduced emergence of *Haematobia irritans* less than 7%, in comparison with the emergence of about 72.50% in the control treatments

Table 2. Egg mortality of C. *binotalis* after being applied with entomopathogenic fungi isolates at the rate of 107 conidia mL-1 consentration

Isolate	Egg mortality ± SD (%)
BAP5	51.95 ± 15.63 a
BPP1	26.90 ± 14.38 ab
NAP3	28.42 ± 16.73 ab
MAP1	12.36 ± 18.17 bc
FAP5	23.49 ± 6.91 ab
FPP3	16.88 ± 5.64 b
PPP4	16.04 ± 8.09 b
Control	0.00 ± 0.00 c

Value(s) followed by same letter(s) are not significantly different

The effects of entomopathogenic fungi on *C. binotalis* larvae at different stages is presented in Table 3. Mortality percentages were significantly different for all

four stages at day 7 post treatment. The young larvae were more susceptible to all isolates of entomopatogenic fungi tested than were the older ones. The 1st instar larvae were most sensitive to the entomopathogen as mortality reached 70.42%. The 2nd larval stage is less sensitive recording a mortality of 47.08%. *Beauveria* (isolate BAP5 and BPP1), *Nomuraea* (isolate NAP3), *Metarhizium* (isolate MAP 1), *Fusarium* (isolate FPP3 and FPP3) and *Paecilomyces* (isolate PPP4) caused high mortality of *C. binotalis* larvae. *Beauveria* isolate (BAP5 and BPP1) showed the highest mortality rate compared with other treatment. However, there were no significant differences with *Nomuraea* (NAP3), *Metarhizium* (MAP1) and *Fusarium* (FPP3). *Paecilomyces* was less effective compared to other treatments.

The larval mortality obtained in the present study was lower than that observed by other authors such as Nashim *et al.* (2002) who reported that *P. fumosoroseus* at a concentration of 2 x 10^6 conidia mL^{-1} resulted in more than 80% arval mortality. Mortality of 100% was recorded at $2X10^7$ conidia mL^{-1}. Tanada and Kaya (1993) suggested that the differences in virulence among the isolates was due to differences in the ability to produce enzymes and mycotoxins during the passage of an infection process in insects such as contact with the cuticle and within the hemocoel. Virulent isolates had a higher enzyme activity compared with avirulent isolates. The higher mortality of *B. salubricola* in the first and second instars compared to the fourth instar is due in part to the cuticle's hydrophobicity, which in the first instars favours spore adherence and consequently increases host susceptibility to fungal propagules (Boucias et al., 1988). Other defense mechanisms include those performed by hemocytes, free cells circulating in the hemolymph, the number and types differ between species, age and stage of development (Gupta, 1985).

Table 3. The mortality percentage of C. *binotalis* after treated with entomopathogenic fungi at a concentration of 107 conidia mL^{-1}

Isolates	Mean mortality of larval stages (%)				Mean mortality of larvae (%)
	1	2	3	4	
BAP5	90.00	66.67	43.33	43.33	60.83 a
BPP1	86.67	70.00	20.00	50.00	56.67 a
NAP3	90.00	63.33	26.67	26.67	51.67 ab
MAP1	83.33	56.67	30.00	33.33	50.83 ab
FAP5	80.00	46.67	26.67	36.67	47.50 abc
FPP3	60.00	46.67	20.00	43.33	42.50 bc
PPP4	73.33	26.67	20.00	26.67	36.67 c
Control	0.00	0.00	0.00	0.00	0.00 d

Value(s) followed by same letter(s) are not significantly different

The shortest time needed to produce 50% mortality (median lethal time, LT_{50}) was dependent on isolates and larval stage. The lethal time to 50% mortality (LT_{50}) values for first to fourth instar larvae of *C. binotalis* ranged between 1.47–3.74 d, 3.66 – 6.07 d, 6.76 – 13.21 d and 5.82 – 10.66 d, respectively. *Fusarium* (FPP3) and *Paecilomyces* had the lowest virulences against *C. binotalis* with an LT_{50} of 3.74 – 17.01 d and 1.80 – 13.82 d, respectively (Table 4).

Table 4. LT_{50} values of different stages of C. *binotalis* after treated with entomopathogenic fungi

Isolates	LT_{50} value (days) of different stage of *C. binotalis* larvae			
	1	2	3	4
BAP5	1.71 (1.27 – 2.10)	4.34 (3.80 – 4.98)	6.76 (5.54 – 9.68)	6.94 (5.73 – 9.86)
BPP1	2.17 (1.59 – 2.70)	4.10 (3.58 – 4.71)	13.21 (8.73 – 65.70)	5.82 (4.99 – 7.34)
NAP3	1.34 (0.80 – 1.79)	3.66 (2.98 – 4.47)	8.55 (7.06 – 14.19)	10.66 (7.47 – 28.72)
MAP1	1.47 (0.87 – 1.96)	4.31 (3.44 – 5.72)	8.41 (6.90 – 13.56)	10.03 (6.87 – 27.60)
FAP5	2.24 (1.63 – 2.79)	6.07 (4.50 – 8.37)	9.86 (7.32 – 21.59)	8.70 (6.45 – 17.39)
FPP3	3.74 (2.68 – 5.46)	6.18 (4.99 – 8.90)	17.01 (9.57 – 179.82)	6.61 (5.70 – 8.53)
PPP4	1.80 (0.86 – 2.53)	13.82 (8.16 – 95.15)	9.82 (7.70 – 24.51)	10.30 (7.41 – 25.01)

Longer LT_{50} values for *C. binotalis* larvae infected by *B. bass*iana is due to the fact that the fungus requires to complete several steps from infection to kill the insects, which starts from attachment of conidia on the insect body, germination, penetration, invasion and colonization in hemocoel, tissues and organs. Time for each of these stages varies depending on the type of fungus isolate, host insect and environment (Neves and Alves, 2004). Conidia which landed on the cuticle germinated within four to six hours. The infected larva then entered the moribund stage at 12 to 24 h after inoculation. Death of the larva occurred after 24 to 28 h (Nashim *et al*. 2002).

Effect of essential oils against *A. ipsilon*

Antifeedant activity (choice test)

Data in Fig. 1 showed varying degrees of antifeedant activities in different treatments against *A. ipsilon*. The essential oils of lavender, mint, patchouli and nutmeg had highly significant antifeedant properties on the third instar larvae of *A. ipsilon*. The percentage antifeedant activity reached more than 50%, while other treatments showed only moderate antifeedant effects (3.70 – 40.00%). These results are in agreement with the finding by Monache *et al*. (1984) who isolated an active component from *M. rigida* root extracts which showed a high degree of antifeedant activity against *Pieris brassicae* and *Locusta migratoria*. The natural products

nortriterpene quinone methides (pristimerin, tingenonee and 20-alpha-hydroxytingenone) were isolated from *Maytenus* sp. (Celastraceae) and their effects were tested on larvae of codling moth (*Cydia pomonella*). Extracts of *M. myristica* were effective in controlling *Podagrica* spp. in Okra (Emosairue and Uguru, 2001). It is also reported to be effective against *Callosobruchus maculatus*. (Dales 1996). Myristicin is a poisonous constituent of nutmegs . The other constituents include charicol, thymol and a-pinene (Dales 1996). These might constitute repellent and/or antifeedant factors which inhibited *C. sordidus.* Citronellal did not demonstrate any significant feeding deterrence on *S. litura* (Hummelbrunner and Isman 2001)

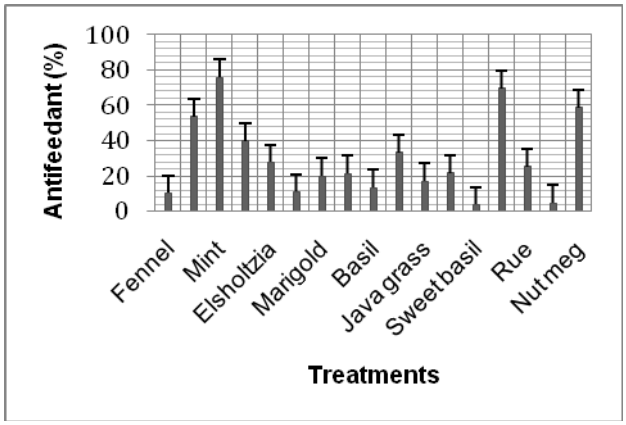

Fig. 1. Antifeedant activity of different treatments against third instar larvae of A. ipsilon (mean ± SE) in choice test

Effect of essential oils of aromatic plants on some biological aspects of 3rd instar larva of A. ipsilon

Results given in Table 5 indicated that there were significant differences between the essential oils tested; the essential oils of different plant had differential activity on *A. ipsilon*. Larval mortality was relatively higher in case of essential oils of pomelo, marigold, pencil pine, basil, cinnamom, java grass, sweet basil, patchouli, and rue, which caused >70% mortality. Pupal mortality was recorded in basil oil, finger root and patchouli oil (> 60%), while mint and nutmeg caused the highest pupal mortality (90%). These results showed that mint and nutmeg oil have a latent effect on larvae of *A. ipsilon* up to certain limit while pupal mortality was affected with these oils. The pupal weight (male and female) were highly significantly reduced in all essential oils tested as compared with the control. The most effective essential oils in reducing the pupal weight were patchouli, nutmeg and basil. There were no significant differences among the essential oils tested on pupal duration compared with the control, except for fennel oil as it recorded only six days while that of control showed 12 d.

Longevity of adult: All tested oils significantly reduced the longevity of adults, including pre oviposition and oviposition periods. Pomelo oil, pencil pine and lavender were more effective in reducing the adult longevity than other treatments. Schmutterer (1990), who found that the adult longevity of many insect species was shortened after application of higher concentrations of neem extracts or azadirachtin. The effect was more pronounced at the higher concentrations. Abd El-Rady and Osman (2005) mentioned that Neemix reduced the adult longevities of *A. ipsilon* compared with control and this may be due to the reduction in their weights and inhibition of proteins, lipids and carbohydrates (Table 5).

Fecundity: The result of Table 6 and Fig. 2 showed that the essential oils of tested aromatic plants significantly reduced the number of eggs laid per female compared to the control. Mint, basil, pomelo, fennel, pencil pine and lemon grass essential oils recorded the highest effect in decreasing the fecundity of *A. ipsilon* more than 50%, while sweet basil and java grass showed the lowest effect on the *A. ipsilon* fecundity. Similar results were reported by Momen *et al.* (2001) that mint reduced the total number of eggs laid by *Tetranychus urticae.*

Egg hatchability: All the essential oils used had a significant effect on the percent egg hatchability of *A. ipsilon*. The highest reduction in egg hatchability was recorded for fennel oil, lavender, basil and cinnamon oil (100%) followed by rue and pencil pine recording about 99.72% and 90.70%, respectively. Mint and Elsholtzia gave a moderate reduction in egg hatchability, with 62.54% and 55.93%, while the other treatments were on par with the control. This reduction in egg hatchability may be due to physiological disturbance in the hormonal system of adults when fed as larvae on treated leaves (Shadia *et a*l. 2007), toxicity of the oil vapors to eggs (Schmidt *et a*l. 1991), or attributed to some chemical ingredients present in the volatiles of tested oils which probably diffused into eggs and affected the vital physiological and biochemical processes associated with embryonic development as recorded by Gurusubramanian and Krishna (1996) and Moawad (2000).

Table 5. Biological effects of 17 essential oils of aromatic plants against *A. ipsilon* larvae

Treatments	Larval Mortality	Pupal weight (g)		Pupal duration	Pupal Mortality
		Male	Female		
Fennel (*F. vulgarae*)	40.00 e	0.31	0.31	6.00 b	60.00 c
Lavender (*L. angustifolia*)	37.50 e	0.30	0.22	11.00 a	51.29 c
Mint (*Mentha* sp)	47.50 d	0.23	0.24	10.00 a	90.00 a
Citronella grass (*C. nardus*)	52.50 d	0.27	0.18	10.50 a	23.33 e
Elsholtzia (*E. pubescens*)	52.50 d	0.21	0.27	14.00 a	43.33 d
Pomelo (*C. grandis*)	72.50 c	0.30	0.24	12.00 a	54.29 c
Marigold (*T. erecta*)	75.00 b	0.27	0.24	14.00 a	50.00 c
Pencil pine (*C. sempervirens*)	72.50 c	0.27	0.21	13.00 a	46.25 d
Basil (*O. smericanum*)	85.00 a	0.23	0.22	14.00 a	65.00 b
Cinnamon (*C. verum*)	82.50 a	0.22	0.32	13.00 a	56.67 c
Java grass (*C. winterianus*)	85.00 a	0.28	0.25	12.00 a	52.50 c
Finger root (*B. pandurata*)	65.00 c	0.26	0.26	13.00 a	62.73 b
Sweet basil (*O. basilium*)	72.50 c	0.34	0.23	14.00 a	56.67 c
Patchouli (*P. cablim*)	80.00 a	0.11	0.13	10.00 a	61.43 b
Rue (*R. graviolens*)	80.00 a	0.29	0.28	14.00 a	23.33 e
Lemon grass (*C. citratus*)	67.50 c	0.33	0.29	10.00 a	23.33 e
Nutmeg (*M. fagrans*)	35.00 e	0.22	0.22	14.00 a	90.00 a
Control	0.00 f	0.45	0.41	12.00 a	0.00 g

Value(s) followed by same letter(s) in a column are not significantly different

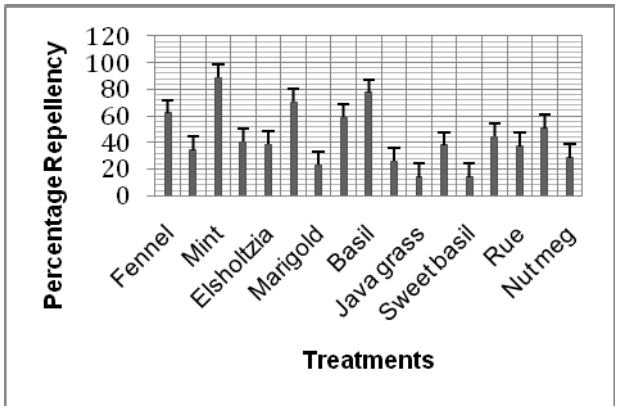

Fig 2. The effect of essential oils on percentage repellency (mean ± SE) of *A. ipsilon.*

CONCLUSION

The entomopathogenic fungus *Beauveria bassiana* (BPP5) is highly pathogenic to *C. binotalis*. Spore concentrations of 10^7/ml for *B. bassiana* showed considerable potential as a microbial control agent for the management of *C. binotalis*. The total percent mortality of eggs and hatched larvae was 51.95%, and larval mortality of 50% was achieved in 1.71 – 6.94 days. Among the essential oil tested, lavender, mint, patchouli and nutmeg showed high potential as antifeedants. Mint, basil, pomelo, fennel, pencil pine and lemon grass had highest effects in decreasing the fecundity of *A. Ipsilon*, and the highest reduction in egg hatchability was recorded in fennel, lavender, basil, cinnamon, rue and pencil pine. Strong insecticidal activity was recorded in lavender, mint, basil, fennel and pencil pine and most of them have a repellent action, reduced fecundity and decreased egg hatchability. These results suggest that naturally occurring entomopathogenic fungi and essential oils of aromatic plants could be useful for managing *C. binotalis* and *A. ipsilon* on cabbage in Indonesia

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Table 6. Oviposition inhibitory effects of 17 essential oils of aromatic plants against A. ipsilon moths on cabbage

Treatments	Pre-oviposition	Oviposition periods	Adult longivity	Mean No. of eggs/female	Egg Hatchability	Reduction in egg hatchability %
Fennel (*F. vulgarae*)	3.00 d	6,50 abc	11,50 d	1173.67ef	0.00 c	100.00
Lavender (*L. angustifolia*)	3.67 abc	7.00 abc	9,50 f	2036.33cd	0.00 c	100.00
Mint (*Mentha* sp)	4.67 a	5.00 abc	13.00 ab	353.00 g	34.00 b	62.54
Citronella grass (*C. nardus*)	3.67 cd	8,33 abc	11,50 d	1837.33cd	76.59 a	15.61
Elsholtzia (*E. pubescens*)	3.33 dc	7.00 abc	11.00 e	1897.33cd	40.00 b	55.93
Pomelo (*C. grandis*)	4.00 a	4,33 bc	8,50 g	910.00fg	84.74 a	6.63
Marigold (*T. erecta*)	3.33 cd	6,33 abc	10.00 e	2380.33bc	83.13 a	8.41
Pencil pine (*C. sempervirens*)	3.00 d	7.00 abc	9,50 f	1278.00ef	5.56 c	90.70
Basil (*O. americanum*)	4.00 a	7.00 abc	11,50 ed	695.33fg	0.00 c	100.00
Cinnamon (*C. verum*)	1.67 e	7.00 abc	12.00 cd	2294.33bc	0.00 c	100.00
Java grass (*C. winterianus*)	2.00 e	10.00 a	12.00 cd	2664.00 a	81.75 a	9.93
Finger root (*B. pandurata*)	3.00 d	9,33 ab	12,50 ab	1923.67cd	87.92 a	3.13
Sweet basil (*O. basilium*)	4.00 a	5,33 abc	12.00 cd	2659.00ab	86.75 a	4.23
Patchouli (*P. cablim*)	2.33 f	3,33 c	12.00 cd	1727.00de	86.84 c	4.32
Rue (*R. graviolens*)	3.00 d	7,33 abc	11,33 d	1940.67cd	0.25 c	99.72
Lemon grass (*C. citratus*)	4.33ab	5.00 abc	9.00f	1520.67de	86.25 a	4.97
Nutmeg (*M. fragrans*)	3.67 bcd	7,33 abc	12,33 ab	1209.67ef	77.77 a	14.00
Control	3.33 cd	10.00 a	13,33 a	3094.00 a	90.76 a	-

Value(s) followed by same letter(s) in a column are not significantly different

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Efficacy of *Plutella xylostella* parasitoids in South Africa and their use in biological control – a review

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ABSTRACT

The pest status of the diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) in South Africa is lower than in other countries with similar climates. A rich indigenous parasitoid fauna of 34 species in total has been recorded to attack all developmental stages of the pest, often reaching parasitism rates of above 90%. In addition, a large number (175) of wild Brassicaceae plants recorded from South Africa on which *P. xylostella* can develop, serve as reservoir for *P. xylostella* and its parasitoids. It is proposed that the pest may have originated in the Cape Floral Kingdom of South Africa.

The impact of parasitoids on *P. xylostella* populations was studied using the insecticide check method to evaluate the efficacy of the parasitoids, and the level of control achieved by them. The results showed that population levels of *P. xylostella* on treated plants were significantly higher than on the untreated plants, and that parasitism of larvae and pupae was significantly higher on untreated plants. On sprayed plants parasitism fluctuated around 5-10%, whereas parasitism levels increased to >90% on untreated plants towards the end of the season. This demonstrated the importance of parasitoids in curtailing *P. xylostella* populations.

On St. Helena Island, South Atlantic Ocean, *P. xylostella* was a serious pest, and farmers were heavily dependent on chemical insecticides to control the pest. The only parasitoid of *P. xylostella* found on St. Helena was *Diadegma mollipla* (Holmgren) (Hymenoptera: Ichneumonidae) which was unable to reduce pest populations. *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae) and *Diadromus collaris* Gravenhorst (Hymenoptera: Ichneumonidae) were introduced into the island from South Africa. Before releases of parasitoids farmers were encouraged to stop all chemical insecticide applications, and spray *Bt* only if necessary to give the parasitoids chance to survive. Within two years of release both parasitoids had become well established throughout the Island and, since then, infestations have remained so low that chemical control has been unnecessary.

As past explorations for natural enemies for biological control of *P. xylostella* in tropical and subtropical counties focused on Europe, it is suggested that more attention be given to biotypes of parasitoids from South Africa and other regions with climates similar to the target countries.

Keywords

Plutella xylostella, biological control, insecticide check method, South Africa, St. Helena Island

INTRODUCTION

The diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae), is the most injurious insect pest of brassica crops throughout the world and occurs wherever these crops are grown (Talekar and Shelton 1993). It is the most universally distributed insect of all Lepidoptera and has the ability to migrate and disperse over very long distances (Meyrick 1928). In many countries, *P. xylostella* has become resistant to every synthetic insecticide used against it in the field (Talekar and Shelton 1993) and also to the bacterial insecticide *Bacillus thuringiensis* Berliner (*Bt*) formulations (Tabashnik et al. 1990, Liu et al. 1995). The increasing usage of *Bt* products resulted in an increasing number of reports of field resistance by *P. xylostella* populations (Tabashnik 1994). The pest also has developed cross-resistance and multiple-resistance to different chemical pesticides (Shelton et al. 2000). Lack of effective natural enemies, especially specific parasitoids, is considered to be the main cause for its high pest status in most parts of the world (Lim 1986). In many countries, in addition to the development of resistance, the destruction of natural enemies by the widespread use of broad-spectrum insecticides is also considered responsible for the imbalance (Talekar and Shelton 1993).

Numerous parasitoids and predators attack all developmental stages of *P. xylostella*. In addition, general predators such as birds and spiders often consume adult moths. Over 90 species of parasitoids have been recorded worldwide (Goodwin 1979) attacking all developmental stages of *P. xylostella*. Of these, the most predominant and effective larval parasitoids belong to three major genera, *Apanteles, Cotesia,* and *Diadegma* and pupal parasitoids belonging to the genus *Diadromus*. For biological control of *P. xylostella* some parasitoid species have been introduced to Southeast Asia, the Pacific Islands, North and Central America, Africa, the Caribbean, Australia and New Zealand with various degrees of success (Lim 1986).

Plutella xylostella is a pest of brassica crops in South Africa (Annecke and Moran 1982) but its pest status is lower than in other countries with similar climates (Kfir 1998). Its biology was studied in South Africa by Gunn (1917) and the mortality factors by Ullyett (1947). Ullyett recorded parasitoids, predators, bacteria and an entomopathogenic fungus associated with it and, at the time, concluded that *P. xylostella* was well controlled by its natural enemies in South Africa. Later Dennill and Pretorius (1995) demonstrated that high infestation levels by *P. xylostella* are a result of excessive insecticide applications. At one study site where insecticides were

applied only once every three weeks, parasitism of *P. xylostella* reached 90% and the pest did not cause economic losses. In contrast, at a second study site with regular and excessive chemical applications, parasitism levels were negligible and serious outbreaks of *P. xylostella* caused total crop losses. Other studies in the Eastern Cape, Gauteng and North-West Provinces of South Africa revealed very high parasitism levels of *P. xylostella* in unsprayed cabbage crops (Kfir 1997a, 1997b, Waladde et al. 2001, Smith and Villet 2002) whereas in the same regions economic damages were recorded by farmers who regularly sprayed their cabbage fields. This indicated that insecticides interfered with the natural control of *P. xylostella* in South Africa. During these studies a total of 34 species have been identified as being associated with *P. xylostella* in South Africa (Kfir 1997a, 1997b, 2003, Lohr and Kfir 2004).

Table 1. Parasitoids recorded from *Plutella xylostella* in South Africa and their importance

Family/ species name	Host relationship	Importance[1]	Source
Hymenoptera: Braconidae			
Apanteles halfordi Ullyett (*A. eriophyes* Nixon)	2nd-3rd instar	**	Kfir, 1997a, 2002
Chelonus curvimaculatus Cameron	egg-larval	*	Kfir, 1997a
Chelonus ritchiei Wilkinson	egg-larval	*	Ullyett, 1947
Chelonus sp.	egg-larval	*	Kfir, 1997a
Cotesia plutellae (Kurdjumov)	2nd-3rd instar	****	Kfir, 1997a
Cotesia (Apanteles) ruficrus Haliday	Larvae	*	Ullyett, 1947
Cotesia sp	2nd-3rd instar	*	Kfir, 2003
Habrobracon brevicornis (Wesmael)	2nd-3rd instar	*	Kfir, 1997a
Hymenoptera: Ceraphronidae			
Aphanogmus fijiensis (Ferrière)	hyper	*	Kfir, 1997a
Hymenoptera: Chalcididae			
Brachymeria sp. 1	hyper	*	Kfir, 1997a
Brachymeria sp. *2*	Pupae	*	Kfir, 1997a
Hockeria sp.		*	Kfir, 1997a
Proconura sp.	Hyper	*	Kfir, 1997a
Stomatocera sp.	pupae	*	Ullyett, 1947
Hymenoptera: Eulophidae			
Oomyzus sokolowskii (Kurdjumov)	3rd instar – prepupae; facultative hyper	***	Kfir, 1997a
Tetrastichus howardi (Olliff)	pupae	*	Kfir, 1997b
Tetrastichus sp.	hyper	*	Kfir, 1997b
Hymenoptera: Eurytomidae			
Eurytoma sp.	hyper	*	Kfir, 1997b
Hymenoptera: Ichneumonidae			
Diadegma mollipla (Holmgren)	2nd-4th instar; Larval-pupal	***	Kfir, 2002
Diadegma sp. 1	Larval-pupal	*	Ullyett, 1947
Diadegma sp. 2	Larval-pupal	*	Kfir, 1997a
Diadromus collaris Gravenhorst	pupae	***	Kfir, 1997a
Mesochorus sp	hyper	**	Kfir, 1997a
Hemiteles sp.	hyper		Ullyett, 1947
Itoplectis sp. 1	Larval-pupal	*	Kfir, 1997a
Itoplectis sp. 2	hyper	*	Ullyet 1947
Macromalon sp. 1	Larval-pupal	*	Kfir, 2003
Macromalon sp. 2	hyper	*	Ullyett, 1947
Hymenoptera: Perilampidae			
Perilampus sp.1	Hyper	*	Ullyet 1947
Perilampus sp. 2	hyper	*	Ullyet 1947
Hymenoptera: Pteromalidae			
Pteromalus sp.	hyper	**	Kfir, 1997a, Kibaboul, 1996
Diptera: Tachinidae			
Carducia plutellae Emden	larvae	*	Ullyett, 1947
Peribaea sp.	larvae	*	Kfir, 1997a

Parasitoid importance[1]: * very rare, ** rare, *** abundant, **** major mortality factor

The Mediterranean region has been suggested as the area of origin for *P. xylostella* (Hardi 1938, Harcourt 1954). This was based on the assumption that the pest evolved

on *Brassica* plants in Europe, and that it had been distributed accidentally around the world with the cultivated brassicas. Kfir (1998) challenged this hypothesis by suggesting a South African origin for *P. xylostella*. This was based on the diversity of wild crucifer plants and the numerous parasitoids of *P. xylostella* recorded in South Africa. This paper reviews the **e**fficacy of the indigenous parasitoids of *P. xylostella* in South Africa and their use in biological control.

The origin of *P. xylostella*, and its parasitoids in South Africa

The complex of parasitoids of *P. xylostella* in South Africa is richer than in other regions of the world with similar climates. Altogether, 34 species of parasitoids and hyperparasitoids have been recorded from larvae and pupae of *P. xylostella* in South Africa indicating a very long association between parasitoids and this insect in the region. Table 1 lists all parasitoids recorded from this pest in South Africa and their importance as natural enemies of *P. xylostella*. Altogether 3 egg-larval parasitoids, 7 larval parasitoids, 6 larval-pupal parasitoids, 4 pupal parasitoids and 13 hyperparasitoids have been recorded. By far the most abundant was the larval parasitoid, *Cotesia plutellae* (Kurdjumov), which was responsible for about 80% of parasitism followed by the larval-pupal parasitoids, *Diadegma mollipla* (Holmgren) and *Oomyzus sokolowskii* (Kurdjumov), the pupal parasitoid *Diadromus collaris* Gravenhorst and the larval parasitoid *Apanteles halfordi* Ullyett. The most abundant hyperparasitoids were *Pteromalus* sp. and *Mesochorus* sp. (Kfir 1997a, 1997b). *Oomyzus sokolowskii*, the only known gregarious parasitoid of *P. xylostella*, acted as a facultative hyperparasitoid, occasionally emerging from cocoons of *C. plutellae* and *A. halfordi*. However, its activity as a primary parasitoid far exceeded its hyperparasitic activity (Kfir 1997a, 1997b). A point of interest is that in Hungary *O. sokolowskii* has been recorded as an obligatory hyperparasitoid attacking *Diadegma* spp only (Melika et al. 2006). This is an indication that a different biotype of this species occurs in Europe. *Apanteles halfordi* (*A. eriophyes* Nixon) is specific to *P. xylostella* and occurs only in South Africa (Walker and Fitton 1992).

In contrast to the large number of parasitoids of *P. xylostella* in South Africa, only a few parasitoids were recorded in regions where the insect is exotic. In New Zealand, for example, only two parasitoids, *Diadegma laterallis* (Grav) and *Diadromus* sp., which together achieved negligible parasitism, were recorded before the introduction of *Diadegma semiclausum* Hellen from Europe (Muggeridge 1930, Robertson 1939). Similarly, *Tetrastichus howardi* (Olliff), was the only parasitoid of *P. xylostella* present in the vegetable-producing areas of Malaysia before the introductions of other parasitoids (Talekar and Shelton 1993).

The Cape Floral Kingdom in the southern part of South Africa is the smallest and richest of the six floral kingdoms of the world. It has the richest plant species diversity in the world with 8600 species—more than 10% of all known flowering plants—all crammed into a 46,000 square kilometer area (Arnold and De Wet 1993). A total of 175 wild plant species in the Brassicaceae have been recorded in South Africa, of which only 32 are naturalized (Jordaan 1993). *Plutella xylostella* develops mainly on plants from this family, all of which contain mustard oils.

The rich and diverse fauna of *P. xylostella* parasitoids in South Africa and the large number of indigenous crucifer plants on which *P. xylostella* can develop are indications that *P. xylostella* may have originated in South Africa.

Efficacy of *P. xylostella* parasitoids in South Africa

To assess the effect of parasitoids on levels of infestation by *P. xylostella*, trials were set up in cabbage fields in the North-West and Gauteng Provinces of South Africa using the insecticide check method (Kfir 2004). A selective insecticide, dimethoate, an organophosphate compound with systemic and contact action, was used to suppress the natural enemies. In trials with cotton pests in California, it was shown that dimethoate is detrimental to natural enemies but causes very little harm to Lepidoptera larvae (Eveleens et al. 1973, Ehler et al. 1973).

The results of the trials showed that at all sites the levels of *P. xylostella* infestations on the insecticide treated plants were significantly higher than on the untreated plants. On the other hand, the level of parasitism of larvae and pupae of *P. xylostella* throughout the season was significantly higher on the unsprayed plants (Kfir 2005). On the sprayed plants parasitism levels fluctuated between 5 and 10% whereas on the control plants parasitism increased rapidly to above 90% towards the end of the season (Kfir 2004).

These results of the trials demonstrated that the higher infestation levels of cabbage by *P. xylostella* in the insecticide-treated plots were caused by partial elimination of parasitoids, and that those parasitoids play an important role in curtailing populations of *P. xylostella* on cabbage crops.

The use of *P. xylostella* parasitoids from South Africa in biological control

Until recently *P. xylostella* was a severe pest of cole crops on the island of St. Helena, South Atlantic Ocean. Because the recommended dose of insecticides failed to control the pest, farmers on St. Helena sprayed higher dosages and even cocktails of several insecticides in attempts to control *P. xylostella.* Surveys in cabbage fields on St. Helena revealed that only one parasitoid, *D. mollipla*, attacks *P. xylostella* on the island but it did not reduce the pest to below economic damage level (Kfir and Thomas 2001). To alleviate the situation a biological control project was hence initiated and ARC-PPRI of

South Africa was assigned to supply natural enemies of *P. xylostella*. In addition, local personnel in St. Helena were trained in rearing and release techniques and how to monitor parasitoid dispersal and establishment in the field (Kfir and Thomas 2001).

To complement the island resident larval-pupal *D. mollipla* parasitoid, a larval parasitoid, *C. plutellae*, and a pupal parasitoid, *D. collaris*, were introduced into St. Helena. The parasitoids were sent by ship as there is no airport on the island. The parasitoids were mass reared locally but before field releases extension officers advised local farmers to use only the more selective *Bt* sprays and refrain from using broad spectrum chemical insecticides to give the newly released parasitoids the best possible chance of survival.

Large numbers of *C. plutellae* and *D. collaris* were then released on ten different farms across the island during 1999 and 2000 (Kfir and Thomas 2001). Follow-up surveys between 2001 and 2004 revealed that both parasitoids became established, and that they gradually dispersed to adjacent fields until they were eventually found to be present throughout the island. As a result populations of *P. xylostella* have collapsed and remained low even in spring (September-October), which is when *P. xylostella* outbreaks usually occurred on St. Helena. Moreover, cocoons of *C. plutellae* were collected throughout the island, indicating that parasitoids had played a major role in the decline of the pest populations. *Plutella xylostella* infestations remain low and no insecticides have been necessary since 2001. This is a strong indication for the success of the biological control of *P. xylostella* on St. Helena (Kfir 2005).

Cotesia plutellae has been recorded to occur in East Africa but the parasitoid is extremely rare in that region (Lohr, personal communication). In 2006 the highly efficient *C. plutellae* biotype from South Africa was introduced into quarantine of the International Centre for Insect Physiology and Ecology (ICIPE) in Kenya. Here, the parasitoid was mass reared, and released in cabbage plots in Uganda and Tanzania. The parasitoid became well established and dispersed widely from the original release fields to adjacent sites (B. Lohr and B. Nyambo, personal communications). Initial surveys indicated that the project had been a success but unfortunately the *P. xylostella* programme at ICIPE has been scaled down, and there has been no further follow up on this project.

DISCUSSION

The hypothesis that *P. xylostella* originated in the Cape Floral Kingdom of South Africa contradicts the widely accepted view that the pest evolved in the Mediterranean region of Europe, and that it spread further with cultivated brassicas around the world. This hypothesis has implications for biological control of *P. xylostella*. The most effective natural enemies of an insect are normally present in its area of origin. Thus, past explorations for *P. xylostella* natural enemies for introductions into other continents focused on Europe. As a result, European natural enemies, e.g. *D. semiclausum*, have been collected in England, introduced into New Zealand and from there to several other countries in Southeast Asia, where *P. xylostella* is a serious pest (Talekar and Shelton 1993). These parasitoids achieved limited control of the pest, and then only in cooler, high elevation regions. They have had negligible effects on the pest population levels at low-lying, warm regions. This is not surprising, because, as expected, European natural enemies are adapted to temperate climate conditions, and struggled to survive in hotter climates.

There is compelling evidence that different biotypes of the principal *P. xylostella* parasitoids exist in different regions of the world. The following examples demonstrate this point: 1. Rincon et al. (2002) studied populations of *C. plutellae* from Martinique, South Africa, Reunion, Taiwan and Benin and found morphological differences between these different populations as well as some reproductive incompatibilities. 2. Lohr and Kfir (2004) reported large differences in color patterns of *C. plutellae* adults, and differences in ecological adaptations and efficiency between biotypes from different parts of the world. The *C. plutellae* biotype in South Africa is a highly efficient parasitoid compared to the very rare *C. plutellae* biotype in East Africa (Lohr and Kfir 2004). 3. In South Africa *Oomyzus sokolowskii* is mainly a primary parasitoid whereas in Hungary it is an obligatory hyperparasitoid (Melika et al. 2006). 4. Birot et al. (1999) compared populations of *O. sokolowskii* from Romania, Pakistan and Benin, and found that crosses between Romanian and Pakistani populations produced significantly lower proportions of female progeny or no progeny at all. There were also differences at isoenzyme levels indicating large differences between the populations. 5. *Diadegma semiclausum* has been reported to be the major mortality factor of *P. xylostella* in the Jordan valley of Israel at 200 m elevation below sea level (M. Coll, personal communication) as well as in the Nile valley of Egypt (A.S. Abdel-Razek, personal communication). There is no doubt that the Israeli and Egyptian biotypes of *D. semiclausum*, which are able to endure the extremely high summer temperatures in these regions, are physiologically different biotypes from the European biotype of *D. semiclausum* which failed to achieve any control of *P. xylostella* in the low-lying, hot regions of Southeast Asia.

The examples presented here indicate that, beyond Europe, there is a potential source of natural enemies that can be exploited for biological control of *P. xylostella*, but which has not yet been tapped.

Recent papers and conference proceedings dealing with *P. xylostella* reveal the trend of a decline in papers on biological control, and an increase in papers on chemical control. It appears that researchers have the perception that, because the existing natural enemies of *P. xylostella* are not efficient and the source of natural enemies has been exhausted, the future of controlling the pest lies with the new chemistry insecticides. However, in the

past, it has been shown repeatedly that *P. xylostella* has developed resistance to all new insecticides that are developed as existing insecticides become useless (Shelton et al. 2000; Nisin et al. 2000). No doubt this trend will continue with the new chemistry insecticides.

As past explorations for natural enemies for biological control of *P. xylostella* focused on Europe, it is suggested that more attention be given to different biotypes of parasitoids from South Africa and other regions of the world with similar climatic conditions to the target countries.

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Naturally-occurring parasitism of diamondback moth in central Iran

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ABSTRACT
The recent major outbreaks of diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae), in crucifers plants in Iran has led to attempts for sustainable pest management strategies, mainly based on natural enemies. The present study aimed to investigate naturally occurring parasitism of *P. xylostella* in cabbage and cauliflower fields of central Iran. For this purpose, field studies were performed to identify parasitoids of *P. xylostella*, and to evaluate percentage parasitism of *P. xylostella* using the recruitment method in main cabbage growing areas of Isfahan province in 2009. In this study, seven species of parasitoid wasps (five larval and two pupal parasitoids) and two species of hyperparasitoid wasps were determined. The parasitoids were included the braconids *Cotesia plutellae* (Kurdjumov), *Bracon hebetor* Say and *Apanteles* sp., the ichneumonid *Diadegma semiclausum* (Hellen), and the eulophid *Oomyzus sokolowskii* (Kurdjumov) as larval parasitoids, and the ichneumonids *Diadromus collaris* (Gravenhorst) and *Diadromus subtilicornis* (Gravenhorst) as pupal parasitoids. In addition, the pteromalids *Mokrzeckia obscura* Graham and *Pteromalus* sp. were identified as the hyperparasitoids, which in turn, parasitize *C. plutellae*. The most predominant species were *C. plutellae* and *D. semiclausum* with the proportional abundance of 0.43 and 0.42, respectively. The species *M. obscura*, *D. collaris* and *Apanteles* sp. are new records from Iran. Percentage parasitism varied significantly between host plants, but not between areas; the parasitized proportion of *P. xylostella* larvae fed on common cabbage was significantly greater than on cauliflower (0.42 vs. 0.34). The mean percentage parasitism varied between 14.5 and 68.4 for different fields, and accounted for 37.4% of *P. xylostella* population on average. The greatest parasitism was achieved by *C. plutellae*, *D. semiclausum* and *O. sokolowskii*, with a parasitism of 21.0, 12.9 and 3.5 percent of field populations of *P. xylostella*, respectively. These findings illustrated the important role of parasitoids for sustainable management of diamondback moth.

Keywords
Plutella, parasitoids, parasitism, recruitment, Iran

INTRODUCTION
The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera, Plutellidae), is the most destructive and cosmopolitan pest of cruciferous plants (Talekar and Shelton 1993). The overuse of chemical pesticides against this pest has resulted in resistance to all groups of insecticides, including insect growth regulators and *Bacillus thuringiensis* Berliner. In addition, the intensive use of insecticides has eliminated effective natural predation of *P. xylostella* in field. Previous studies have shown that larval and pupal parasitoids are the most effective natural enemies of *P. xylostella* (Talekar and Shelton 1993; Sarfraz et al. 2005; Karimzadeh and Wright 2008). In this regard, parasitoids must be the cornerstone of any pest management program looking for *P. xylostella* sustainable management (Verkerk and Wright 1996).

Another key biotic factor in regulation of *P. xylostella* populations in the field is host-plant availability (Kfir 1997). Plants may mediate many of the interactions between herbivores and their parasitoids, influencing the preference and performance of parasitoids (Cortesero et al. 2000). Generally, in a tritrophic system variation in host-plant characteristics may have differential effects on a herbivore and its associated natural enemies (Karimzadeh and Wright 2008). Many studies revealed that different host-plant species or cultivars have differential effects on *P. xylostella* parasitism success by parasitoids, in particular, *Cotesia plutellae* (Kurdjumov) and *Diadegma semiclausum* (Hellen) (e.g., Talekar and Yang 1991; Verkerke and Wright 1997; Haseeb et al. 2001; Liu and Jiang 2003; Karimzadeh et al. 2004; Karimzadeh and Wright 2008).

In recent years, *P. xylostella* has shown major outbreaks in cabbage and cauliflower fields in Isfahan province (central Iran). To overcome such a serious problem, struggling farmers have used all available synthetic insecticides up to more than 10 times of recommended doses. Unfortunately, the overuse of pesticides offers no satisfactory control of the pest, and has increased environmental and health concerns. This study aimed to identify larval and pupal parasitoids of *P. xylostella*, and to assess the natural percentage parasitism of field populations of *P. xylostella* on two different host plants.

MATERIALS AND METHODS

The study sites

The study was carried out in two main cabbage growing areas of Isfahan province (Iran), which were located in Falavarjan (32° 33′ N; 51° 31′ E) and Mobarakeh (between 32° 3′ N and 32° 28′ N; between 51° 13′ and E 51° 48′ E) counties.

Parasitoid species and their abundance

To identify the larval and pupal parasitoids of *P. xylostella*, in each county two fields of common cabbage (*Brassica oleracea* var. *capitata*) cv. Globe Master and two fields of cauliflower (*B. oleracea* var. *botrytis*) cv. Royal were selected. Each field was sampled fortnightly between June (one month after transplantation) and October (harvest) 2009. Sampling was carried out on ten randomly selected plants within each field, where all *P. xylostella* 2nd, 3rd and 4th instar larvae, prepupae and pupae on each plant were collected and reared under laboratory conditions (25 ± 5 °C, 70 ± 5% RH and L:D 16:8 h). Emerged parasitoids were then identified by Gavin Broad (Department of Entomology, The Natural History Museum, London, UK), Hosseinali Lotfalizadeh (Department of Plant Protection, East Azerbaijan Research Center for Agriculture and Natural Resources, Iran), Jeno Papp (Department of Zoology, Hungarian Natural History Museum, Budapest, Hungary), John LaSalle (Division of Entomology, Commonwealth Scientific and Industrial Research Organization (CSIRO), Canberra, Australia), James B. Whitfield (Department of Entomology, School of Integrative Biology, School of Life Sciences, College of Liberal Arts and Sciences, University of Illinois, Urbana, USA), Kees van Achterberg (Department of Terrestrial Zoology, National Museum of Natural History, Leiden, Netherlands) and Mark R. Shaw (National Museums of Scotland, Edinburgh, UK). The number of individuals for each parasitoid species was recorded and used as an index for abundance.

Percentage parasitism

Recruitment method (van Driesche et al. 1991) was used to evaluate natural parasitism of *P. xylostella* larvae. The sampling was carried out during August to October 2009. In each area, two fields of common cabbage and two fields of cauliflower were chosen such that there was a minimum of 5 km distance between the fields. No pesticide was applied in the selected fields from one week before to end of sampling process. Sampling was then carried out on ten randomly selected plants within each field; all the 2nd, 3rd and 4th instar larvae, prepupae and pupae of *P. xylostella* were collected and transferred to the laboratory. In the laboratory, only the 4th instar larvae were reared under standard constant conditions (25 ± 5 °C, 70 ± 5% RH and LD 16:8 h) and all other stages were discarded. A larva reared on the related host plant until the moth had pupated or the parasitoid cocoon had formed (here the number of formed parasitoid cocoons) was recorded as parasitism success (Karimzadeh et al. 2004). After 48 h, the same plants were searched and the number of 2nd instar larvae recruited to plants was recorded (as the recruited hosts). The whole process was repeated one week later in the same fields. The rate of parasitism was calculated as the ratio of the parasitism success to the recruited hosts (Verkerk and Wright 1997).

Statistical analyses

Differences in the level of parasitism rate between host-plant types and between regions were analyzed using logistic analysis of deviance (binomial errors). All statistical analyses were completed in R.2.10.0 (Crawly 2005, 2007).

RESULTS AND DISCUSSION

Larval and pupal parasitoids of P. xylostella and their abundance

In present study, seven species of parasitoid wasps (five larval and two pupal parasitoids) and two species of hyperparasitoid wasps were determined (Table 1). Larval parasitoids were three braconids, *C. plutellae* (Kurdjumov), *Apanteles* sp. and *Bracon hebetor* Say, an ichneumonid, *D. semiclausum* (Hellen), and a eulophid, *Oomyzus sokolowskii* (Kurdjumov). Pupal parasitoids were ichneumonids *Diadromus collaris* (Gravenhorst) and *D. subtilicornis* (Gravenhorst). The hyperparasitoids were pteromalids *Mokrzeckia obscura* Graham and *Pteromalus* sp. that act as the parasitoids of *C. plutellae*. This is the first record of *M. obscura*, *D. collaris* and *Apanteles* sp. on *P. xylostella* in Iran. The most predominant species were *C. plutellae* and *D. semiclausum* with the proportional abundance of 0.43 and 0.42, respectively (Table 1).

Natural percentage parasitism of P. xylostella

There was a significant difference (t_{157} = -3.339, P < 0.01) between host plants for the mean percentage parasitism; the mean percentage parasitism of *P. xylostella* larvae fed on common cabbage was significantly greater than on cauliflower (42.8 vs. 33.7). However, no significant difference was observed between areas (t_{156} = 0.797, P = 0.43; Table 2). In addition, comparison of sampling times showed that mean percentage parasitism in second sampling time was significantly greater (t_{157} = 3.810, P < 0.001) than that in first one (42.8 vs. 32.3). The mean percentage parasitism varied between 14.5 and 68.4 for different fields, and accounted for 37.4 percent of *P. xylostella* population on average (Table 2). The greatest parasitism was achieved by *C. plutellae*, *D. semiclausum* and *O. sokolowskii*, with a parasitism of 21.0, 12.9 and 3.5 percent of field populations of *P. xylostella*, respectively.

Table 1. Parasitoids of *P. xylostella* and their abundance in Isfahan Province, Iran.

Parasitoid			Occurrence				
					Area (number)‡		
species	Family	Type	Time	Plant †	F	M	Total
Cotesia plutellae	Braconidae	larval	May – Oct.	CA, CO	212	255	467
Apanteles sp. *	Braconidae	larval	Sept.	CA	0	5	5
Bracon hebetor	Braconidae	larval	Oct.	CO	0	3	3
Diadegma semiclausum	Ichneumonidae	larval	May – Oct.	CA, CO	176	279	455
Diadromus subtilicornis	Ichneumonidae	pupal	July – Oct.	CA, CO	5	10	15
Diadromus collaris *	Ichneumonidae	pupal	July – Oct.	CA, CO	8	0	8
Oomyzus sokolowskii	Eulophidae	larval-pupal	June – Oct.	CA, CO	86	39	125
Mokrzeckia obscura *	Pteromalidae	hyperparasitoid	Sept.	CA	0	2	2
Pteromalus sp.	Pteromalidae	hyperparasitoid	Sept.	CA	0	1	1

* **The species is recorded for first time from Iran.**
† **Host-plant type: CA = cauliflower, CO = common cabbage.**
‡ **County: F = Falavarjan, M = Mobarakeh.**

Table 2. Natural parasitism of *P. xylostella* by larval parasitoids in Isfahan Province, Iran.

Sampling				Percentage Parasitism (Mean ± SE)		
County	Host plant	Field	Time			
Falavarjan	Common cabbage	1	1st	29.4 ± 6.7		
			2nd	51.5 ± 8.3		
		2	1st	49.5 ± 10.7		
			2nd	30.4 ± 6.1	40.2 ± 4.3	
	Cauliflower	1	1st	14.5 ± 4.8		
			2nd	41.1 ± 8.2		
		2	1st	32.5 ± 5.5		
			2nd	43.2 ± 9.1	31.2 ± 4.0	34.9 ± 2.9
Mobarakeh	Common cabbage	1	1st	37.8 ± 4.1		
			2nd	68.4 ± 8.8		
		2	1st	36.1 ± 5.8		
			2nd	36.8 ± 4.3	42.8 ± 3.6	
	Cauliflower	1	1st	31.9 ± 4.8		
			2nd	42.4 ± 3.4		
		2	1st	29.3 ± 3.2		
			2nd	28.2 ± 3.8	35.3 ± 2.1	38.9 ± 2.2

The results indicate that despite numerous applications of insecticides against diamondback moth in cabbage fields of Isfahan province, the diversity and performance of parasitoids are noticeable. The level of natural parasitism under current situation (high pressure of pesticides and no support for biological control agents) is fascinating, implying that biological control plays a key role in suppressing *P. xylostella* populations. In this regard, it is necessary to support naturally occurring parasitism in fields by limiting pesticide use. Furthermore, mass rearing and release of effective parasitoids such as *C. plutellae*, *D. semiclausum* and *Diadromus* spp. accompanied with more environmentally friendly pesticides such as *B. thuringiensis* might be complementary to natural check by parasitoids. It also is essential to evaluate parasitism level accurately during several subsequent growing seasons in each area to have a better understanding of natural check by parasitoids

Both of the host plants used in this study have shown to be partially resistant to attack by *P. xylostella* (Jafary et al. 2010). The observed differences in parasitism between these two host plants, therefore, might be due to biochemical differences between host plants. The induced volatiles from infested plants vary between plant species or cultivars; such volatiles can cause special behavior in parasitoids (Vet and Dicke 1992).

Several studies have demonstrated that two specialist larval parasitoids of diamondback moth, *C. plutellae* and *D. semiclausum*, have different responses to various crucifer species or cultivars (Talekar and Yang 1991; Verkerke and Wright 1997; Liu and Jiang 2003; Karimzadeh et al. 2004; Rossbach et al. 2006). Apart from the mechanisms underpinning the plant-mediated differences in natural parasitism of *P. xylostella* (Bukovinszki et al. 2005; Kahuthia-Gathu et al. 2008; Karimzadeh and Wright 2008), such difference can be useful for manipulating crop-pest-parasitoid system to enhance the effects of parasitoids.

CONCLUSION

Isfahan province has a high potential for biological control of *P. xylostella*. Naturally occurring parasitism must be supported and improved. This cannot be practicable unless the pressure of chemical pesticides is limited and studies focus on sustainable management strategies based on native parasitoids.

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Diversity and abundance of diamondback moth parasitoids in north Thailand

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ABSTRACT
Diamondback moth (DBM), *Plutella xylostella,* poses a major constraint to crucifer vegetable production in the highlands of northeastern Thailand. Heavy reliance on chemical control resulted in frequent DBM resurgence, human health hazards, environmental pollution, and threat to the export market. To overcome these problems, biological control was explored, with initial surveys to determine the parasitoid diversity and abundance. These were conducted on crucifer crops above 1000 m altitude in Chiangmai and Phetchabun highlands.

Findings revealed no DBM egg parasitoid but presence of larval (*Cotesia plutellae*, *Macromalon orientale*) and pupal (*Diadromus collaris*) parasitoids, with average overall combined parasitism of 42.3%. Larval parasitoids contributed 22.6% parasitism and pupal parasitoid 19.7%. *Cotesia plutellae* was dominant in early crop stages, while *M. orientale* and *D. collaris* dominated during later stages. Their abundance has no clear relationships with temperature and relative humidity.

Parasitism was generally higher in organic and IPM fields surveyed than in fields with farmers' practices, indicating frequent pesticide applications by farmers (sometimes 18-20 times per cropping cycle) were detrimental to parasitoids. That higher DBM populations were also associated with intensively-sprayed fields suggests the parasitoids do exert an influential role on DBM.

Past experiences indicated parasitism of at least 70% over extended period being necessary to sustain full DBM control. But the existing parasitoid complex could achieve only 42.3% total parasitism, suggesting the species as inherently ineffective to provide full control of DBM, either individually or combined. This is further supported by field observations and farmer feedback that DBM continues to be crucially important and most difficult to manage.

The surveys failed to recover *Diadegma semiclausum*, confirming that an earlier introduction effort into Phetchabun was unsuccessful. Since *D. semiclausum* has exhibited superior performance in many highlands elsewhere, the best next option would be to re-introduce it to supplement the existing parasitoid complex.

Keywords
Diamondback moth, parasitoids, biological control, Thailand

INTRODUCTION
Thailand, an agricultural country, has increased substantially its vegetable production for both domestic and export markets over the past two decades. In particular, the expansion has involved the cultivation of non-indigenous crucifer vegetables, such as head cabbage (*Brassica oleracea*), Chinese kale and cauliflower in the cooler northern highlands. In parallel, there is also an increase in frequent and serious outbreaks of *Plutella xylostella* or the diamondback moth (DBM). Applying chemical insecticides is the common method used to deal with DBM. Although the amount of pesticides applied has been increasing year by year, outbreaks of DBM have continued unabated and are causing heavy crop losses.

Over the years, the excessive use of insecticides, especially broad-spectrum ones, have posed severe problems, such as environmental contamination, escalating costs in production, and human health hazards from direct spray exposure by farmers and indirectly from consumption of contaminated vegetable produce by consumers. More recently, stricter international trade regulations and food safety standards are threatening Thailand's vegetable export markets. Consequently, there is great concern over the suitability of the pest control approach, which relies exclusively on chemical pesticides. This is especially so because the Government is promoting Thailand to be "the kitchen of the world" through good agricultural practices (GAP) and making efforts to ensure that all produce is of the highest safety and health quality. To support this, at least 96 kinds of pesticides have been banned since 2004, and all produce must pass the "test for residues" and be certified safe prior to sale.

In practice, the challenge for farmers is to grow more with less pesticide use, and with less hazardous pesticides. But farmers are constrained by a lack of choice of selective and less toxic pesticides, while at the same time pests (in particular DBM) continue to be abundant. Unless an alternative means is available, this

approach would continue until it eventually becomes completely unsustainable.

Fortunately, there exists a promising alternative strategy, which is harnessing effective biological control agents. For DBM, the parasitoid wasp, *Diadegma semiclausum*, has proven to be highly effective in many of the cool tropical highlands. In Thailand, introducing this exotic wasp into the Khaokor highlands of Phetchabun province was jointly attempted in the mid-1990s by the Department of Agriculture (DOA) and the Asian Vegetable Research and Development Center (AVRDC). However, the wasp is believed to have failed to establish although official documentation confirming this is lacking.

Recently, the Thai Government has expressed a renewed interest to explore the potential of *D. semiclausum* for the management of DBM in crucifer production systems in the northern and northeastern highlands. A standard procedure in biological control is to first survey the existing local biodiversity prior to introductions. The Department of Agriculture Extension (DOAE), with support from FAO and DANIDA, initiated this task, and this report describes the studies undertaken and the findings obtained.

The overall objective of the surveys is to determine the parasitoid biodiversity and abundance in relation to DBM on crucifer crops in the northern and northeastern highlands of Thailand, and specifically to establish:

- What DBM parasitoids are present;
- What are the levels of parasitism;
- What are their distributions;
- How their populations relate to DBM;
- Whether *D. semiclausum*, which was previously introduced into the northeast highlands in Phetchabun province, has actually failed to establish, or possibly remained undetected.

MATERIALS AND METHODS

The studies were carried out in both the Chiangmai and Phetchabun highlands. In Chiangmai, the lowlands were excluded because a survey on DBM and its parasitoid complex had earlier been undertaken by Rowell et al. (2005). The current studies in the higher elevations therefore also serve to complement their studies.

In the present studies in Chiangmai province and Maehongsorn province, six cabbage fields spread over different parts of the highlands were surveyed. They have differing conditions in terms of pesticide input levels. Selection of the different conditions was to help understand how the parasitoids would perform under the different pesticide regimes as follows:

- Field 1 (or Maerim 2): Organic farm in Maerim district (Chiangmai province) with no use of chemical pesticide.
- Field 2 (or Hod 2): IPM treatment field in a Farmer Field School (FFS) in Hod district (Chiangmai province) with no chemical pesticide used. For pest management, farmers used only biorational products (e.g. neem, Bt, or nematode) and cultural practices or mechanical methods.
- Field 3 (or Sobmouey): Similar to Field 2, except located in Sobmouey district (Maehongsorn province).
- Field 4 (or Hod 1): Conventional farmer field in Hod district where chemical pesticides were sprayed frequently. Number of sprays for Fields 4, 5, and 6 ranged from 18-20 times per season.
- Field 5 (or Hod 3): Another conventional farmer field in Hod district.
- Field 6 (or Maerim 1): Conventional farmer field in Maerim district (Chiangmai province).

In northeastern Phetchabun province (where *D. semiclausum* was previously released but believed to have failed in establishing), the surveys were done in two fields at Khaokor and Lomkao districts.

For the surveys, all the sites were selected based on the following criteria: altitude at least 1000 m above sea level, local cropping systems predominantly crucifers with year-round production, and participating farmers agreeing to stipulated planting conditions (especially not using chemical pesticides). From each survey site, as many as possible of DBM eggs, larvae and pupae, and cocoons of the DBM parasitoid *C. plutellae*, were collected weekly (until crop harvest) and reared separately in the laboratory to determine what ultimately would emerge:

- From DBM eggs to: (1) DBM larvae, or (2) adult egg parasitoids, or (3) nothing emerging.
- From DBM larvae to: (1) DBM pupae (and later to DBM adults, or adults of larval-pupal parasitoids, or nothing emerging), or (2) cocoons of larval parasitoids (and later to parasitoid adults, or other adults of hyper-parasitoids, or nothing emerging), or (3) nothing emerging.
- From DBM pupae to: (1) DBM adults, or (2) adults of pupal parasitoids, or (3) nothing emerging.
- From *C. plutellae* cocoons to: (1) the adult parasitoids, or (2) other adults of hyper-parasitoids, or (3) nothing emerging.

The surveys were carried out from August-December (2004) in Chiangmai province and Maehongsorn province, and from January-February (2005) in Phetchabun province. The weather parameters, temperature and relative humidity were also recorded.

RESULTS AND DISCUSSION

In Chiangmai province and Maehongsorn province, both the sites Maerim 2 (organic farm) and Sopmouey (IPM field) had neglible DBM populations (Fig. 1). These were in sharp contrast to all the farmer practice fields

(Maerim 1, Hod 1 and Hod 3) where DBM infestations were about five folds more. Hod 2, an IPM field, also had high DBM populations.

With respect to egg parasitoid of DBM, none was found in all the survey sites in the provinces of Chiangmai, Maehonsorn and Phetchabun (Table 1).

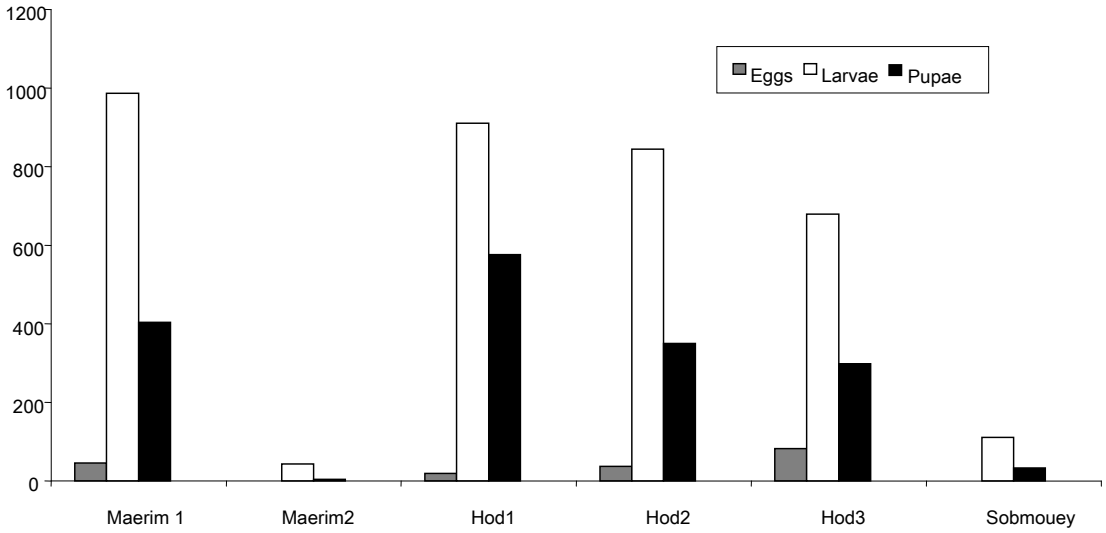

Figure 1. DBM populations in survey sites of Chiangmai province and Maehongsorn province.

Table 1. Status of DBM eggs in the study sites

Site	Total (No.)	Hatched (%)	Parasitized (No.)
Maerim 1	45	100	0
Maerim 2	0	-	-
Hod 1	19	100	0
Hod 2	37	86.5	0
Hod 3	82	97.6	0
Sobmouey	0	-	-
Khaokor	18	100	0
Lomkao	15	100	0

No egg parasitoid was found even though *Trichogrammatoidea bactrae* has previously been released for DBM control in the lowland areas of Amphur, Sarapee and Chiangmai province (AVRDC 1993). Also, in a study undertaken from 1985-89, Keinmeesuke et al. (1992) had found *T. bactrae* in the lowland areas of Nakornprathom province and Kanchanaburi province and *Trichogramma confusum* in the highland areas of Phetchabun province. A possible reason for not finding egg parasitoids (should they be present) in the current surveys is because the overall number of eggs that could be found was relatively few.

Pertaining to DBM larvae collected, two kinds of parasitoids were found from surviving larvae, namely *C. plutellae* and *Macromalon orientale*. Both parasitoids were found in all the study sites. Total combined parasitism varied from 14.8% to 37.6% (Table 2.) In general, the parasitism was higher in organic and IPM fields than in the farmer practice fields. On average, mortality of the larvae from other causes was about 10%.

Table 2. Parasitism of DBM larvae in the study sites

Site	Total collected (No.)	Surviving larvae (No.)	Parasitized larvae*	
			No.	(%)
Maerim 1	987	858	274	31.9
Maerim 2	43	37	13	35.1
Hod 1	910	839	124	14.8
Hod 2	845	706	147	20.8
Hod 3	679	556	93	16.7
Sobmouey	111	101	38	37.6
Khaokor	437	437	117	26.8
Lomkao	751	751	162	21.6

*Combined total parasitism for *C. plutellae* and *M. orientale.*

In the case of DBM pupae, only the parasitoid *Diadromus collaris* was recovered. Out of a total of 2,326 pupae collected, its parasitism level varied from 2.1% in Khaokor to a maximum of 40.3% in Lomkao (Table 3). This parasitoid was found in all the sites surveyed. On average, pupal mortality due to other causes was 9.7%.

During the surveys, *C. plutellae* cocoons were also collected to assess the adult emergence rate and the level of hyper-parasitism. The results obtained indicated that adult emergence was particularly high, averaging 94.7% (Table 4). Hyper-parasitism (species not identified) was generally low, averaging only 3.9%. The highest was

recorded in Hod 3 (19.1%), followed by Hod 1 (7.4%). Of the other six fields, none was found in three fields (Maerim 2, Sobmouey and Khaokor), while hyper-parasitism was negligible in the rest (ranging from 0.8-2.5%). For *C. plutellae* cocoons, mortality from other causes was in general insignificant, averaging only 1.4%.

Table 3. Parasitism of DBM pupae in the study sites

Site	Total Pupae No.	Dead No. (%)	No. (%) Pupae parasitized*	
			Yes*	No
Maerim 1	404	41 (10.2)	77 (19.1)	286 (70.8)
Maerim 2	4	0 (0.0)	1 (25.0)	3 (75.0)
Hod 1	576	65 (11.3)	44 (7.6)	467 (81.1)
Hod 2	350	49 (14.0)	106 (30.3)	195 (55.7)
Hod 3	298	64 (21.5)	60 (20.1)	174 (58.4)
Sobmouey	33	6 (18.2)	10 (30.3)	17 (51.5)
Khaokor	281	0 (0.0)	6 (2.1)	275 (97.9)
Lomkao	380	0 (0.0)	153 (40.3)	227 (59.7)

*Pupae parasitized by *D. collaris* only.

In terms of timing of the parasitoid dominance, the situation in Hod 2 is selected for illustration, primarily because (i) more sample points were taken from this field (hence can give a clearer picture of the parasitoid occurrence) and (ii) there was no hazardous chemical insecticide applied (consequently no disruption of the natural occurrence of the parasitoids). The findings indicate that in general the larval parasitoid, *C. plutellae*, was dominant in the earlier crop stage (Fig. 2). Its population, however, declined in the later crop stage when both the larval parasitoid *M. orientale* and the pupal parasitoid *D. collaris* dominated. Concerning the main weather parameters (temperature and relative humidity), no clear relationship was found to exist with the occurrence or dominance of the parasitoids. Many reports in the past have indicated DBM to be among the most important pests of crucifer crops in the cooler highlands of Thailand (Keinmeesuke et al. 1992; Rowell et al. 2005). Field observations and farmer feedback in the present surveys have confirmed that DBM is still very important and difficult to manage.

Although DBM abundance is generally governed by the crop stage and normally peaks in mid-season, this often is influenced by farmers' pest management practices. For example, very high DBM infestations may continue late into the season when farmers apply pesticides intensively. This was especially evident in the case of Hod 1 and Hod 3 where the farmers sprayed 18-20 times throughout the season.

IPM strategy with strong biological control focus is currently the best option to overcome pesticide overuse by farmers as exemplified by situations in the region (e.g. Cameron Highlands in Malaysia, Cordillera Highlands in the Philippines and Da Lat highlands in Vietnam). In these locations, DBM, once a serious problem, is now kept under control (with little or no use of insecticides) by parasitoids (especially *D. semiclausum*) in fields where farmers practice IPM or organic farming.

Table 4. Status of *C. plutellae* cocoons in the study sites

Site	Total Cocoons No.	Dead* No. (%)	Adults emerged No. (%)	
			Cotesia plutellae	Others**
Maerim 1	119	1 (0.8)	115 (96.6)	3 (2.5)
Maerim 2	7	0 (0.0)	7 (100.0)	0 (0.0)
Hod 1	68	3 (4.4)	60 (88.2)	5 (7.4)
Hod 2	128	5 (3.9)	122 (95.3)	1 (0.8)
Hod 3	157	0 (0.0)	127 (80.9)	30 (19.1)
Sobmouey	41	1 (2.4)	40 (97.6)	0 (0.0)
Khaokor	63	0 (0.0)	63 (100.0)	0 (0.0)
Lomkao	205	0 (0.0)	203 (99.0)	2 (1.0)

*Cocoons with nothing emerging.
**Hyper-parasitoids; species not identified.

In the current surveys, three species of DBM parasitoids (*C. plutellae*, *M. orientale* and *D. collaris*) were confirmed present in Thailand's northern highlands. Although quite common, farmers were unaware of them and their potential roles. Until FFSs were undertaken recently, farmers neither understood biological control nor IPM, even though parasitoids (*T. confusum*, *C. plutellae* and *D. collaris*) had earlier been reported (Keinmeesuke et al. 1992). Pesticides thus continued to be used regularly and indiscriminately without regard for the conservation and sustainable utilization of ecosystem services provided by natural biological control (e.g. parasitoids).

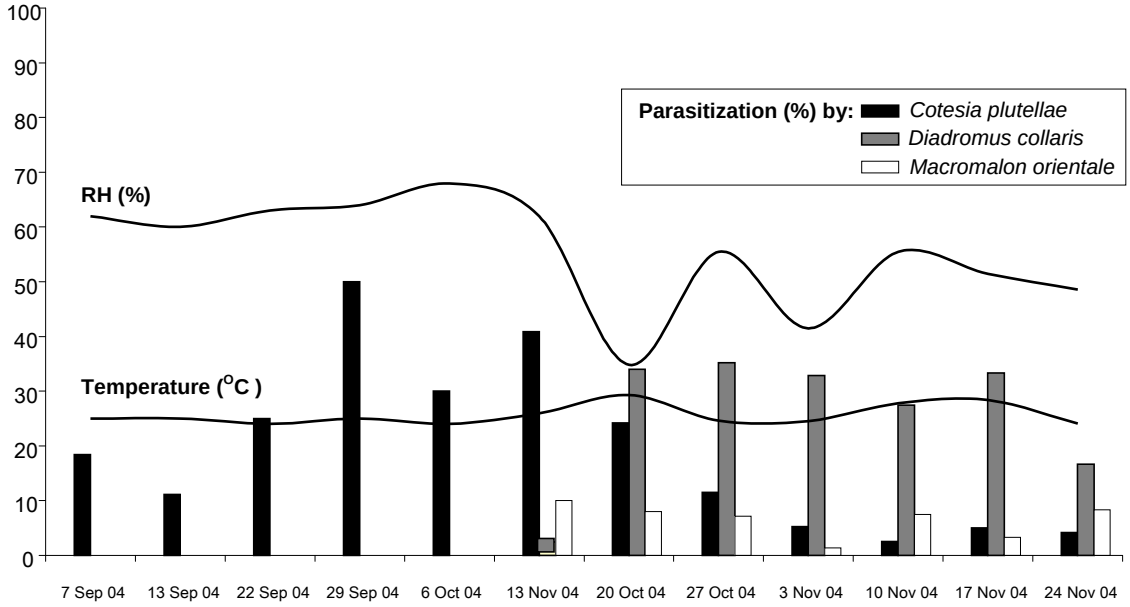

Figure 2. Parasitoid occurrence and dominance over cabbage crop stages in Hod 2 (IPM field), Chiangmai province.

Table 5. Total combined parasitism of DBM by larval parasitoids (*C. plutellae* and *M. orientale*) and pupal parasitoid (*D. collaris*) in Chiangmai, Maehongsorn and Phetchabun provinces.

DBM	No. collected and reared	No. (%) died	No. (%) survived	No. (%) parasitized
Eggs	216	7 (3.2)	209 (96.8)	0 (0.0)
Larvae	4,763	478 (10.0)	4,285 (90.0)	968 (22.6)*
Pupae	2,326	225 (9.7)	2,101 (90.3)	457 (19.7)
Total combined parasitism				42.3%

*Based on surviving larvae.

However, certain fields (e.g. Maerim 1), although sprayed heavily, yet have high levels of parasitoids at the later stage. This, in part, could be due to DBM build-up resulting from pesticide failure because of DBM resistance development, thereby attracting more parasitoids. In such situation, catch-up by the parasitoids is usually too late and cannot provide effective DBM control. That abundant *C. plutellae* also occurred in heavily sprayed fields suggests it probably has developed some resistance. This possibility has been confirmed by bioassay studies in Malaysia (Lim 1982).

Since the average total parasitism from the three parasitoids was only 42.3%, with that in individual field ranging from 31% to 48%, such levels of parasitism are clearly insufficient to provide effective DBM control, even though up to 65% parasitism may occur briefly occasionally and mostly at late crop stages of development. Experiences elsewhere have repeatedly shown that only sustained occurrence of at least 70% parasitism over an extended period, starting at early crop growth stages, can achieve effective DBM control (Lim 1982). Also, this normally occurs in fields where either no chemical pesticide is applied or only selective biological insecticides are used, such as Bt or neem. In the surveys, although such conditions existed in both the organic farm (Maerim 2) and the IPM fields (Hod 2 and Sopmouey), yet the overall combined parasitism was still well below what is needed to give effective and stable DBM control. For instance, although both Maerim 2 and Sopmouey fields have markedly lower levels of DBM population, the Hod 2 IPM field still suffered very high infestation of DBM (Fig. 1). This clearly illustrates that even though there is a complex of at least three parasitoids present in the highlands, these parasitoids are still unable to assure a consistent suppression of the DBM.

Regarding the parasitoid *D. semiclausum* the present surveys did not recover it in both the Chiangmai and Phetchabun highlands. In the former place, the parasitoid cocoons or adults were never observed during the many field visits or from emergence of collected DBM larvae during training of survey staff prior to embarking on the systematic surveys. In Phetchabun highlands where *D. semiclausum* previously had been released by DOA/AVRDC more than a decade ago, failure to find the parasitoid in a thorough search in two fields described below provided strong support to the absence of *D. semiclausum* there.

The first field was a vast area of abandoned cabbage crop while the second field was the left-over of a harvested crop. In both fields, insecticide spraying had stopped a long time ago. In the first field, abundant DBM in all stages was found along with many cocoons of *C.*

plutellae. Many adults of *C. plutellae, M. orientale* and *D. collaris* were also caught, though not a single cocoon or adult *D. semiclausum* was observed. Likewise, the second field was also heavily infested by DBM in all stages (predominantly matured larvae, pupae and adults). *Cotesia plutellae* occurred in very large numbers (every plant examined had it, and many leaves had up to 3 cocoons). Commonly seen flying around or on the plants were adults of the three parasitoids. Again, like the first field, no *D. semiclausum* cocoon or adult was found. In both these fields, should *D. semiclausum* be present, both its cocoons and adults would easily be observed, especially under the conditions where insecticide use had long stopped, so much so that DBM and its other parasitoids (*C. plutellae, M. orientale*, and *D. collaris*) were able to survive and multiply freely and abundantly. That *D. semiclausum* is absent in the Phetchabun highlands inspite of earlier efforts on its introduction is unfortunate, because *D. semiclausum* is the most sought among DBM parasitoids because of its known superior performance in controlling DBM in many parts of the world (Lim 1982; Sastrosiswojo and Sastrodihardjo 1986; Ooi 1992; Talekar et al. 1992; IIBC 1996).

CONCLUSION

Since the complex of parasitoids (*C. plutellae, M. orientale* and *D. collaris*) now existing in both the Chiangmai and Phetchabun highlands can provide only partial control of DBM, the latter will certainly continue to pose a serious problem to crucifer production there. Farmers will persist in spraying heavily to deal with the problem, unless a more effective alternative becomes available to them. As such, it would be desirable that another effort to introduce *D. semiclausum* to supplement the existing parasitoid complex for DBM control in the highlands be undertaken. Only when such effort is successfully achieved can crucifer farming in the highlands be improved to support the Government's vision to promote Thailand as "the kitchen of the world" through good agricultural practices to produce vegetables with the highest safety and health quality.

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Species characterization of microsporidia isolated from lepidopteran pests in Malaysia

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ABSTRACT
The microsporidia from *Plutella xylostella* (PX) and *Spodoptera litura* (SL) were characterized based on microscopic and molecular studies. Microscopically, the type and size of spores were recorded. The severity of infection was also observed histologically. The amplification of SSUrRNA gene using PCR was conducted to see differences in gene sequence of these two microsporidia. Results showed that the type and size of spores as well as the sequencing of the SSUrRNA genes from microsporidium of *P. xylostella* (m-PX) are different from that of *S. litura* (m-SL). The m-PX spores were ovi-cylindrical and larger size (3.167 ± 0.21 µm x 1.61 ± 0.115 µm (n = 35) than m-SL, which was oval, 3.00 ± 0.10 x 2.41 ± 0.11 µm (n = 35) in size. Phylogenetic analysis results indicate that the SSUrRNA gene sequences of m-PX and m-SL spores were in the same clade with other SSUrRNA gene sequences *of Nosema*/*Vairimorpha*. Based on this morphological and molecular data it can be said that the spores of both m-PX and m-SL originated from the same genus, i.e., *Nosema,* but from different species. Failure to confirm the presence of the octospores in infected tissues provided further evidence that the m-PX spores are from the genus *Nosema* which is close to *Nosema plutellae*. On the other hand, the m-SL spores are close to *Nosema bombycis*. The m-PX spores were equally infective to both SL and *Spodoptera exigua* (SE) but with different patterns of infection. SL larvae were more susceptible to microporidium infection from m-PX than SE as indicated by high mortality and severely infected tissues. Results also showed that m-PX spores were concentrated in the epithelial cells of the intestine although they can also be found frequently in the body fat, ganglion and gonads.

Keywords
Microsporidia, *Nosema, Vairimorpha, Plutella xylostella, Spodoptera litura*

INTRODUCTION
Microsporidium is a pathogenic parasite for many insects including lepidopteran pests. It is an obligatory in nature which has a wide distribution around the world. It has a big potential as a microbial pesticide or biopesticide agent for controlling insect populations (Sajap 2004). This parasitic protist has a complex life cycle that often involves more than one host and more than one type of spore (Undeen and Cockburn 1989). There are two modes for transmission used by the parasite in its life cycle, i.e horizontal or vertical transmission (Goertz et al. 2007) by which its spores get transmitted between hosts. Once the spores enter the body, their sporoplasms and nuclei are pushed out into the host's cells through filament tubes to start a new cycle (Dall 1983).

The microsporidium genus *Nosema* is characterized by development in direct control with host cell cytoplasm, diplokaryotic nuclei throughout development and disporous sporogony. The genus *Vairimorpha* exhibits the same features as *Nosema* plus an octosporous sporogony producing uninucleate spores in a sporoporous vesicle. In 1999, Canning et al. conducted an identification study on microsporidia isolated from *Plutella xylostella* (also known as diamondback moth or DBM) from Cameron Highlands, Malaysia. Observation on the sporogonic stages of the microsporidia has been conducted by using light and electron microscopy. Octosporoblastic sporogony was found to be common in this parasite but consistently abortive. The presence of this stage suggested this microsporidium to be referred as *Vairimorpha imperfecta* n.sp. On the other hand, Zainal-Abidin et al. (2004) named microsporidia isolated from Malaysia's *P. xylostella* as *Nosema bombycis* based on the morphological characters. Experimental studies have shown that this parasite can cause high mortality rates in DBM in particular the larval stages (Zainal-Abidin et al. 2004).

This study was carried out to: 1. characterize microsporidia isolated from *P. xylostella* from Cameron Highlands through morphological identification and molecular approach. 2. Determine the pattern of infections and the ultimate effect(s) of the parasite (in laboratory conditions) on another two lepidopteran pest species, *i.e*. *Spodoptera exigua* (SE) and *Spodoptera*

litura (SL) which normally live in the same vicinity as DBM.

MATERIALS AND METHODS

Sample

PX and SL larvae were collected from cruciferous vegetable farms in Cameron Highlands, Pahang, Malaysia, and the third larval instars of SE and SL were provided by the Ministry of Agriculture, Research and Development Institute (MARDI) Malaysia for *in vivo* study.

Morphological identification of m-PX and m-SL

Isolation, purification and observation of spores

The PX and SL larval instars were macerated and ground in a mortar before adding distilled water. The resulting crude suspensions of spores (m-PX and m-SL) were filtered with muslin cloth to remove larval tissues. The suspensions were then centrifuged at 1000g at 10^0C for 10min. The pellets of m-PX and m-SL were resuspended in TE buffer and the spores were purified by mixing with Percoll 90% (1:1) and centrifuged by using gradient centrifuge at 3000g, for 30min, at 4^0C (modification of Tsai et al 2003). The spore concentration was determined by using haemacytometer according to Undeen (1997). Types of m-PX and m-SL spores were determined by observing fresh and giemsa staining slides of the spores through light microscope at 400× and 1000× power of magnification, and the size was measured using a micrometer.

Molecular identification of m-PX and m-SL

PCR amplification and sequencing

Spore suspension (2×10^8 spores in 0.25ml TE buffer) mixed with equal volume of glass beads (0.4mm) in 10 × 75mm glass tubes were shaken at maximum speed on a vortex mixer for 1 min. The homogenate was incubated with proteinase K (mixed with 300µl Tris-HCL, pH9.5, 75µl SDS 10% and 25µl 2-mercaptoethanol 0.1%) for 1 hour at 56^0C to release the DNA from the nuclei (modification of Undeen and Cockburn 1989, Tsai et al 2003). The DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions (Huang et al. 2004). The small subunit (SSU)rRNA gene was amplified from each of the m-PX and m-SL isolates using the primer 18f 5'-CACCAGGTTGATTCTGCC-3' and 1537r 5'-TTATGATCCTGCTAATGGTTC-3' designed by Baker et al (1995). PCR amplification was carried out in 25µl, using 100ng DNA, 20 pmol of each primer, 200µm of each dNTP, 50Mm $MgCl_2$ with PCR buffer and 2.5 U of Taq DNA Polimerase (Promega, Madison, WI). The amplification was performed in PTC-100TM Programmable Thermal Controller (MJ Research, Inc.) for 40 cycles, each with the following profile: 94^0C for 1 min, 57^0C for 1 min and 72^0C for 2 min. A 5µl aliquot from each reaction was run on a 1.2% agarose gel to visualize the PCR product. The PCR product with the size about 1.2 kb was purified using QIAquick PCR Purification Kit (Qiagen Inc. USA) according to manufacturer's instructions, and sent to First Base Laboratories Sdn. Bhd. in Shah Alam, Malaysia for sequencing.

Phylogenetic analysis

The microsporidial SSUrRNA gene sequences of the isolates (m-PX and m-SL) and the other 22 SSUrRNA gene sequences (Table 1) were aligned with CLUSTAL X program (Thompson et al. 1997). Sequence homology analyses were performed using BLAST database searches. The sequence from *Nosema bombi* (Accession No. AY741104) was used as an out-group. Phylogenetic analysis based on the resultant alignment was using neighbor-joining algorithm methods for distance analysis (Kimura 2-parameter) with phylogeny analysis using parsimony, PAUP 4.0b8 (Swofford, 1998). One thousand bootstrap replicates were generated to test the robustness of the tree.

Experimental infection of m-PX to SL and SE larvae

All experiments were conducted in an air-conditioned laboratory. Four doses of m-PX: $1x10^2$, $1x10^3$, $1x10^4$ and $1x10^5$ spores/µl were prepared according to Cantwell (1970). Each dose was dispensed on $1x1cm^2$ mustard and placed in 24-well plastic plates. Then the larval instars were allowed to feed on this spore-contaminated mustard as a means of infection. Control larval instars on the other hand were fed with mustard minus the spores. The development and the mortality rate of the larval instars were monitored and recorded for 5 days beginning at 24, 48, 72, 96 and 120 hours post-infection (hpi). The spore concentrations at the stipulated times were also estimated by scarifying a certain number of the infected larval instars.

Final observations were carried out on the 15^{th} day post-infection. Histological and stained-tissue slides of the infected larval instars were also prepared for histopathological findings. Larvae were fixed in Carnoy's fluid, dehydrated in a graded series of ethanol solutions (70%, 80%, 95% and absolute alcohol), cleared in ethanol:butanol (1:1) for 2 hours and absolute butanol for another 2 hours, embedded in Paraplast wax, and 0.7µm sections were stained by Weigert's iron Hematoxylin stain and Eosin (H&E). Prepared slides were observed under the light microscope at 400× and 1000× power of magnification.

Table 1. The SSUrRNA sequences of microsporidia used for phylogenetic analysis

Accession No.	Microsporidia	Host		
		Genera & species	Order	Family
-	m-PX	*Plutella xylostella*	Lepidoptera	Plutellidae
-	m-SL	*S. litura*	Lepidoptera	Noctuidae
AB125666	*Nosema bombycis1*	*Bombyx mori*	Lepidoptera	Bombycidae
AB093008	*N. bombycis2*	*Bombyx mori*	Lepidoptera	Bombycidae
L39111	*N. bombycis3*	*B. mori*	Lepidoptera	Bombycidae
AB125664	*N. bombycis4*	*Bombyx mori*	Lepidoptera	Bombycidae
AY259631	*N. bombycis5*	*Helicoverpa armigera*	Lepidoptera	Noctuidae
AB036052	*N. bombycis6*	*Antheraea Mylitta Drury*	Lepidoptera	Saturniidae
DQ919077	*Nosema sp.1*	*Pieris rapae*	Lepidoptera	Pieridae
AF240352	*Nosema sp.2*	*Hemerophila atrilineata*	Lepidoptera	Choreutidae
AY211392	*Nosema spodopterae1*	*Spodoptera litura*	Lepidoptera	Noctuidae
AY747307	*N. spodopterae2*	*Spodoptera litura*	Lepidoptera	Noctuidae
DQ073396	*Nosema antheraeae*	*Antheraea pernyi*	Lepidoptera	Saturniidae
AY960987	*Nosema plutellae*	*P. xylostella*	Lepidoptera	Plutellidae
AJ012606	*Nosema tyriae*	*Tyria jacobaeae*	Lepidoptera	Arctiidae
U09282	*Nosema trichoplusiae*	*Trichoplusia ni*	Lepidoptera	Noctuidae
AY958071	*Nosema pyrausta*	*Ostrinia nubilalis*	Lepidoptera	Crambidae
AF327408	*Vairimorpha cheracis*	*Cherax destructor destructor*	Decapoda	Parastacidae
AJ0118331	*Nosema granulosis*	*Gammarus duebeni*	Amphipoda	Gammaridae
U26532	*Nosema furnacalis*	*Ostrinia furnacalis*	Lepidoptera	Pyralidae
AF124331	*Vairimorpha sp.*	*P. xylostella*	Lepidoptera	Plutellidae
AJ131646	*Vairimorpha imperfecta* isolate 2	*P. xylostella*	Lepidoptera	Plutellidae
AF240355	*Endoreticulatus sp.* isolate 1	*Bombyx mori*	Lepidoptera	Bombycidae
AY741104	*Nosema bombi*	*Bombus lucorum*	Hymenoptera	Apidae

The two new isolates used in the present study are indicated in bold.

RESULTS AND DISCUSSION

Morphology of m-PX and m-SL

Result showed that the type and size of spores of m-PX were different from that of m-SL. The m-PX spores were ovi-cylindrical and larger in size (3.167 ± 0.21 µm x 1.61 ± 0.115 µm (n = 35) than m-SL, which were oval, 3.00 ± 0.10 x 2.41 ± 0.11 µm (n = 35) in size. One way analysis of variance (ANOVA) supported that m-PX and m-SL were two different spores with *P* value=0.000. Even though researchers reported variant sizes of m-PX and m-SL, all agreed to group these microsporidia under *Nosema*/*Vairimorpha* genus (Table 2).

Table 2. Sizes of m-PX and m-SL from different studies

Microsporidia (referred name)	Size (µm)	Reference
m-PX *(N.plutellae)*	5.381 ± 0.207 × 2.742 ± 0.115 µm (fresh) 5.029 ± 0.071 × 3.814 ± 0.120 µm (stained)	This study
m-PX *(V. imperfecta)*	4.20 ± 0.1 x 2.0 ± 0.1 µm (fresh) 3.50 ± 0.13 x 2.1 ± 0.06 µm (stained)	Canning et al. (1999)
m-PX *(N. bombycis)*	3.23 ± 0.09 x 1.52 ± 0.098 µm	Rosnizar (2001)
m-PX *(Nosema spp.)*	3.96 ± 0.21 x 1.88 ± 0.10 µm	Tsai et al. (2003)
m-PX *(N. bombycis)*	3.1 ± 1.1 x 2.6 ± 0.9 µm (fresh) 2.7 ± 0.8 x 1.7 ± 0.8 µm (stained)	Zainal-Abidin et al. (2004)
m-SL (N. bombycis)	5.034 ± 0.101 µm x 4.929 ± 0.111 µm (fresh) 4.938 ± 0.298 µm x 2.508 ± 0.159 µm (stained)	This study
SL *(Nosema spp.)*	4.46 x 1.64 µm	Sajap (1995)
SL *(N. spodopterae)*	4.00 ± 0.17 x 1.90 ± 0.12 µm	Tsai et al. (2003)

PCR Amplification

Figure 1 shows 18f/1537r primer set produced an amplicon of PCR (1232bp in size) only with the DNA from spores of microsporidia isolates. Both studied amplicons (SL for m-SL and PX for m-PX) are about 1200bp, suggesting these isolated spores are microsporidia.

Phylogenetic Analysis

The SSUrRNAs of microsporidia are highly conserved genes, making it useless in distinguishing between very closely related species, even those that can be distinguished on morphological criteria, but is still useful for genus identification (Canning et al. 1999, Tsai et al. 2003). The gene contains 1232bp and is located between nucleotides 2677-3908 relative to the 5' end of the rRNA gene (Huang et al. 2004). Results showed that the G+C content of the SSUrRNA gene is 33.9%. Phylogenetic analysis suggests that all 24 microsporidia can be divided into two distinct clades: Clade I consists of microsporidia isolated from Lepidopteran only, whilst clade II consists of microsporidia isolated from Amphipoda, Lepidoptera and Decapoda (Figure 2). Both m-SL and m-PX sequences have been grouped together in sub-clade I that contains microsporidia under genus *Nosema*, *Endoreticulatus* and *Vairimorpha*. This condition leads this study to confirm that these two isolates are members of the *N. bombycis* complex and belong to the same genus, i.e., Nosema but from different species (Tsai et al. 2003).

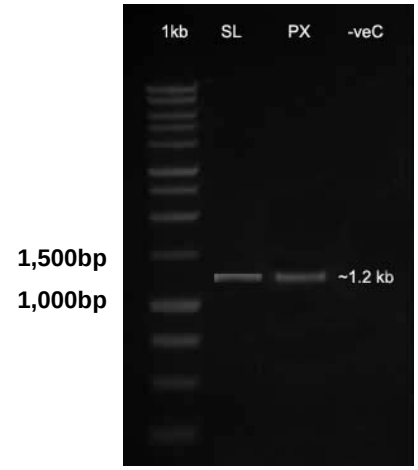

Figure 1. Agarose gel electrophoresis of PCR products. Amplification with the primer set 18f/1537r. Lane 1, 1 kb DNA ladder (Invitrogen). Lane 2, SSUrRNA of m-PX. Lane 3, SSUrRNA of m-SL. Lane 4, negative control (without template)

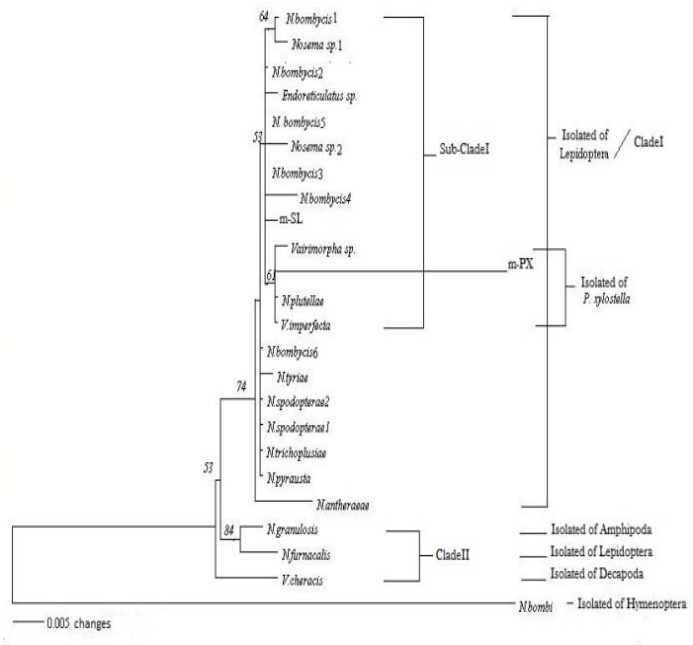

Figure 2. Phylogenetic relationships among microsporidia isolated from various hosts based on SSUrRNA. The tree was generated by the neighbor-joining method using Kimura two-parameter distances

Neighbor-joining (NJ) tree also groups m-PX sequence with the other three microsporidia; *Vairimorpha sp.* (AF124331), *Vairimorpha imperfecta* (AJ131646) and *Nosema plutellae* (AY960987). All of these microsporidia were isolated of *P. xylostella*. Failure to confirm the presence of the octospores in infected tissues provided further evidence that the m-PX spores are from the genus *Nosema* which is close to *Nosema plutellae*.

Based on the sampling location, *Vairimorpha* sp., *V. imperfecta* and *N. plutellae* were isolated from Jerman, Malaysia and Taiwan's *P. xylostella* respectively. This is supported by Solter et al. (2000) who suggest that the specificity of a parasite towards a host is limited by geographical distance. Different species of microsporidia should infect the same species of host in different localities. A study by Ku et al. (2007) on microsporidia isolated from Taipei and Taiwan's *P. xylostella* suggested the parasites be named *N. bombycis* and *N. plutellae* respectively.

All sequences of *N. bombycis*1-6 including m-SL have been grouped together under the same clade 1 (microsporidia isolated from Lepidopteran only) although the strains were isolated from different locations throughout the world. This result suggests m-SL spores are close to *Nosema bombycis*.

Infection of m-PX to SL and SE larvae

The results of this present study are summarized in Figures 3-7 below.

A distinct pattern of infections of m-PX in these two pest species was observed. Until day 5, i.e. 120 hpi, all infected instar groups of SE had an upward trend or increasing degree of infection, especially after 96 hpi (Figure 3) with the presence of many spores in tissues (Figure 7). It seems that spore concentration reached maximum values after 120 hpi. and that the highest dose of infection ($1x10^5$ spores/µl) resulted in the highest spore concentration and caused one death after 96 hpi (Figure 5). All infected instars in all groups succumbed to this infection before or after 15 days post-infection with the majority of them reaching the pupal stage only. On the other hand, control instars were found to have natural infection with the parasite but of a low degree and survived the infection, undergoing metamorphosis to the adult stage successfully. These results indicate that SE were susceptible to m-PX infection and that a low degree of infection in nature was a common phenomenon which normally did not cause death to the insect pests (Chapman and Joern 1990). It was possible that death in the infected instars was due to large number of spores which caused severe damage to tissues (Dunn and Smith 2001).

In infected larval instars of SL, the patterns of m-PX infection were different. Spore concentrations in all groups of infected instars increased drastically until after 72 or 96 hpi, when they declined sharply to minimal levels of less than $2x10^3$ spores/µl (Figures 4 & 6). Control instars also showed the same pattern of infection but with less spore burdens. Interestingly, during this episode of infection, mortality did take place in the infected larval instars as early as 48 hpi, although larval instars also succumbed after 120 hpi and before the 15th day post infection. It was also noted that most of the infected larval instars died at the pupal stage and that control instars managed to transform into adults.

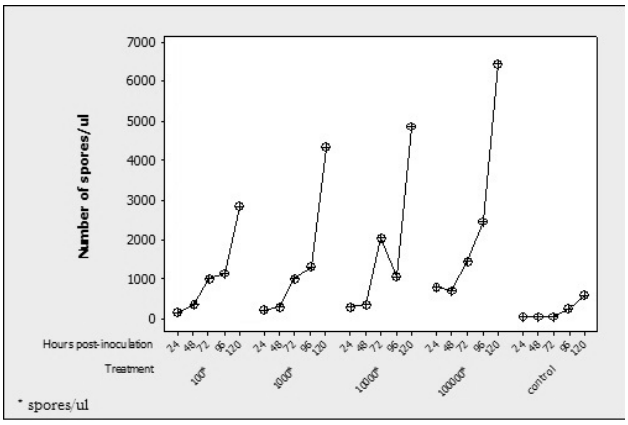

Figure 3. Patterns of infection in SE at 24, 48, 72, 96 and 120 hpi with m-PX

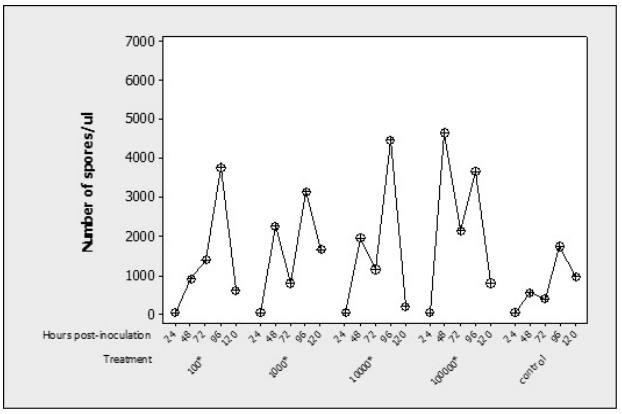

Figure 4. Patterns of infection in SL at 24, 48, 72, 96 and 120 hpi with m-PX

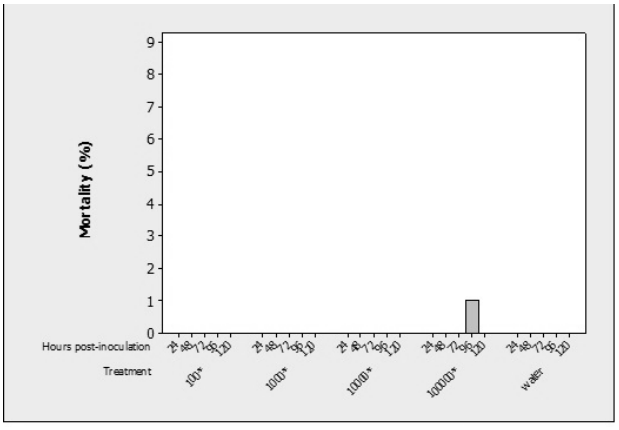

Figure 5. Mortality rate of SE instars during the infection with m-PX

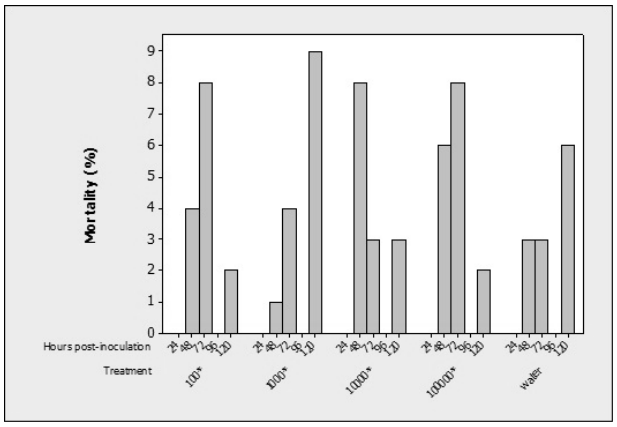

Figure 6. Mortality rate of SL instars during the infection with m-PX

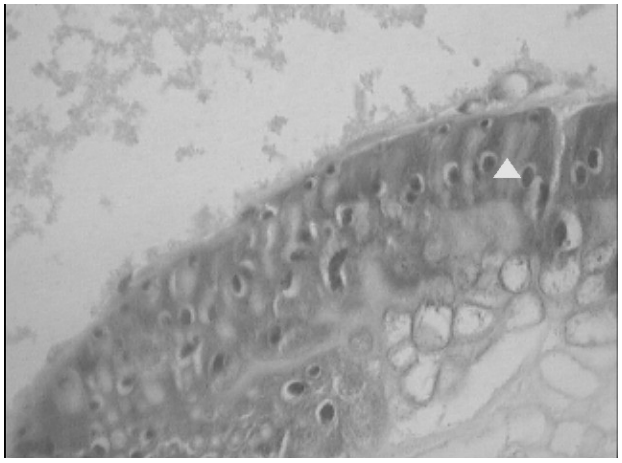

Figure 7. m-PX (arrow) spores in the epithelial cells of the intestine of SE

These results seem to indicate that both pest species are naturally susceptible to m-PX but with different patterns of infection. Upon infection, SL instars managed to mount strong immune response(s) which reduced spore numbers in the body. The question remains as to why they still perish in the course of the infection although they already had reduced parasite numbers. It is possible that there was a breakdown in the immune response(s) (Pang 1986) which allowed the parasite to multiply to great numbers and caused severe tissue damages leading to death. Other factor(s) may also be involved in the death of the infected larval instars.

CONCLUSION

In summary, microsporidia isolated from lepidopteran pests in Malaysia showed host-specific characteristics even though isolated from the same habitat. The ability of m-PX spores to infect SE and SL showed the potential of this parasite to be used as a candidate for a biopesticide for controlling important lepidopteran pests of crucifer crops.

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Effects of temperature and different concentrations of *Nosema bombycis* (Microsporidia: Nosematidae) spores on the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae)

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ABSTRACT
Biological control, using pathogenic microsporidia, may have the potential to be an alternative to chemical control against the diamondback moth (DBM), *Plutella xylostella* (Lepidoptera: Plutellidae). The microsporidium, *Nosema bombyis (NB),* is one of many disease agents that can be used in the Integrated Pest Management (IPM) of DBM. However, its pathogenicity or effectiveness can be influenced by many factors, especially temperature. As such, this study was conducted to discover the effect of temperature on the NB infection of DBM larvae. Infection was performed on second instar larvae at different dose (spore number/concentration) levels (0, 10^2,10^3 10^4,10^5), at temperatures of 20 and 25°C, and at 65% RH, 12:12 (L: D). Larval mortality, adult emergence, fecundity, deformed adults, and the number of normal adults that emerged from the infected DBM were recorded. This was performed on up to the 4th generation of DBM. Results show that there was no significant difference in larval mortality among treatments, although it was somewhat higher at 20°C compared to 25°C. There was a steady decline in the number of eggs and adults from the 1st generation to the 2nd generation, followed by a dramatic decrease in the 3rd and 4th generations for both temperatures; even though the effect of *Nosema* at 20°C on reproduction of *P. xylostella* was more pronounced than at 25°C. However, there was no significant difference in number of deformed adults among treatments (doses) for both temperatures. The optimum temperature for *Nosema sp*. to suppress DBM population could be 20°C and below.

Keywords
Plutella xylostella, Nosema sp, Temperature, IPM

INTRODUCTION
The diamondback moth (DBM) *Plutella xylostella* L. (Lepidoptera: Plutellidae) is a destructive pest of Brassicaceous crops worldwide. In the past several decades, using chemical insecticides for the management of DBM in most countries has led to a rapid build-up of insecticide resistance, and DBM has now reportedly developed a resistance to most of these commonly used chemicals (Tabashnik et al., 1990). However, the ability of *P. xylostella* to develop resistance to insecticides (Tabashnik et al., 1990; Shelton et al., 1993; Zhao et al., 2002), combined with general ecological and health concerns, has encouraged an interest in developing other management techniques, such as the development of Integrated Pest Management (IPM) systems, using bacteria, fungi, viruses, protozoa, endo-larval parasitoids (Ooi, 1992) or nematodes (Mason and Wright, 1997; Somvanshi et al., 2006; Nyasani, 2008) as key components. However, the establishment of these parasitoids into the ecosystem has been adversely affected by the extent of pesticide usage (Talekar et al., 1992). Microbial insecticides, based on *Bacillus thuringiensis (Bt)* and the macrocyclic lactone, Abamectin, have been incorporated into an IPM system for *P. xylostella* (Ooi, 1992; Ibrahim and Low, 1993); but resistance to these bio-insecticides by field populations of *P. xylostella* has already been reported (Tabashnik, 1994; Tabashnik et al., 2008). During the last decade, the fungi *Beauveria bassiana* and *Metarhizium anisopliae* have been tested against DBM, causing rapid larval mortality, which spread quickly within the fields (Yoon, 1999; Godonou et al., 2009), but they were unfavourable due to their slow type of action. Their possible use as control agents of some viruses has also been tried for *P. xylostella* management, with some success (Abdul Kader, 1992).

Nosema bombycis Negali, is one of several important mortality factors of DBM in the field (Idris et al., 2004). DBM mortality was higher in younger instars (1st and 2nd generations) than in the older instars. Even at lower concentrations, infection was also significantly higher for both larvae and pupae in highlands than in lowlands (Idris et al., 2004).

Since temperature is one of the most important ecological factors for the development of insect populations, its effect on the biology of *Nosema* should be studied. Therefore, the purpose of this research is to study the effects of *Nosema* spore concentration on the different stages of DBM, reared at different temperatures. The resulting data will tell us about the kind of climatic conditions within which this pathogen might have more impact in a pest population.

MATERIALS AND METHODS

Source of Insects

The disease-free DBM larvae of the University Putra Malaysia (UPM) strain were provided by the Malaysian Agriculture Research and Development Institute (MARDI). The stock-culture of DBM used throughout this study, were reared on potted cabbage, *Brassica oleracea* var *capitata* in screen cages (38cm x 26cm x 26cm) and maintained at between 25 to 30°C, with a photoperiod of 12L: 12D, and 60 to 80% RH. A 10% honey solution was offered to the adults as food, which had been reared over several generations in a laboratory, prior to experimentation.

Microsporidia

The *Nosema* spores used in the experiments were harvested from the cabbage fields from Cameron Highlands, Pahang. DBM infected with *Nosema* were triturated in a sterilized tissue grinder. This homogenate was partially purified by filtration through a nylon mesh cloth and centrifuged at 3000rpm for 10 minutes. The pellet was re-suspended to 10ml using sterile distilled water, and then the suspension was re-centrifuged. This procedure was repeated three times. The final spore suspension was determined by visual counts with a haemocytometer. Spore suspensions, ranging from 10^2 to 10^5, were prepared by diluting with distilled water, and then stored at 4°C for further study.

Infection of DBM Larvae

Leaf discs (of 2cm diameter) were cut from fully expanded leaves of rape (*Brassica juncea*) plants; that were grown in a greenhouse from seeds. Leaf discs were treated by dipping them for 5 seconds with *Nosema* spore suspensions of different concentrations (0, 10^2,10^3, 10^4, and 10^5). The first group was fed with a piece of leaf dipped in distilled water and used as a control group. After dipping, leaf discs were air-dried at room temperature and placed into wells of 24-cell plastic culture plates, to provide food for 4-6 hour starved 2^{nd} instar larvae; at one larva per well. These wells were put into environmental chambers which was maintained at 20°C or 25°C ± 0.5°C. The relative humidity (70 ± 10%) and photoperiod (12:12 h, light: dark) were kept similar for all the experiments. Larvae were remained on the treated rape leaves for 24 hours. Only the larvae that consumed a complete disc were included in the experiments. Groups of 10 larvae were put into petri dishes and provided with untreated rape leaves in the growth chambers, which maintained at 20 or 25°C ± 0.5°C, (50 larvae per treatment). Fresh untreated rape leaves was supplied to both treated and untreated groups at 24-hour intervals to all dishes. Petri dishes were examined at 24-hour intervals for *P. xylostella* larvae moulting, mortality, and pupation. As soon as the larvae pupaed, they were transferred into labeled sterilized vials. The sex of each emerging adult was determined and the emerged adults from each replication were allowed to mate in laboratory conditions, and the data of their reproduction and mortality were recorded and these was done for four consecutive generations.

Statistical Analysis

All data were subjected to analysis of variance (ANOVA), and treatment means were separated by the Tukey's multiple range tests ($P < 0.05$).

RESULTS AND DISCUSSION

Larval Mortality of *P. xylostella* Inoculated with *Nosema bombycis*

Table 1 shows the cumulative percentage mortality caused by the different spore dosages at 20°C, which ranged from 2 to 60%. Larvae mortality began 1 day after application of *Nosema* and was not significantly different for the first and second days for all concentrations. The mortality of larvae from the third day after treatment and for pre-pupae was significantly different ($p < 0.05$) and was lower in 10^2 and 10^3 than at higher spore concentrations. Larval mortality at 25°C ranged from 0 to 38%, depending on spore concentration (Table 2). The cumulative percentage mortality caused by different concentrations of *Nosema* was not significantly different for the first three days following treatment, but was significantly higher at both the fourth and fifth days; when 10^3, 10^4, and 10^5 spores were used as treatment. However, the highest mortality (at 38%) occurred among larvae exposed to 10^3.

Table 1. Percentage mortality (accumulative) of diamondback moth larvae fed with rape (*Brassica juncea*) leaves at various concentrations of *Nosema* at 20°C.

Nosema doses	Days after treatment					
	1	2	3	4	5	6
Control	2 ± 2a	6 ± 2.5a	10 ± 3.2a	4 ± 2.5a1	18 ± 3.7a	20 ± 5.5a
10^2	6 ± 2.5a	10 ± 3.2a	18 ± 3.7a	28 ± 4.9a	36 ± 5.1a	42 ±6.6a
10^3	12 ± 4.9a	18 ± 3.7a	30 ± 5.5b	40 ±7.1b	50 ± 7.1b	56 ± 9.3b
10^4	6 ± 2.5a	10 ± 3.2a	26 ± 2.5a	36 ± 5.1b	46 ± 9.3b	54± 11.2b
10^5	8 ± 3.7a	14 ± 4.0a	20 ± 5.5a	30 ± 4.5a	56 ± 4.0b	60 ± 5.5b

Means in column with same letters are not significantly different ($P < 0.05$; Tukey).

Table 2. Percentage mortality (accumulative) of diamondback moth larvae fed with rape (*Brassica juncea*) leaves with at various concentrations of *Nosema* at 25°C.

Nosema doses	Days after treatment				
	1	2	3	4	5
Control	0 ± 0a	0 ± 0a	2 ± 2a	4 ± 2.5a	6 ± 2.5a
10^2	2 ± 2a	0 ± 0a	6 ± 4a	10 ± 3.2a	14 ± 4a
10^3	6 ± 4a	8 ± 5.8a	18 ± 6.6a	30± 7.1b	38 ± 5.8b
10^4	2 ± 2a	2 ± 2a	10 ± 3.2a	18 ± 5.8a	20 ± 4.5a
10^5	2 ± 2a	12 ± 3.7a	20 ± 5.5a	26 ± 7.5a	32 ± 10.2b

Means in column with same letters are not significantly different ($P < 0.05$; Tukey).

These findings are in accordance with earlier results, which indicate that DBM larval mortality was high even at lower concentrations of *Nosema* spores (Idris et al., 2004) and for the gypsy moth (Lymantria dispar L.), where mortality varied between 79 and 99%, independent of spore concentration (Goertz et al., 2004).

Egg production, the number of adults emerging, and the number of deformed adults

The effect of five spore concentrations of *Nosema* on the number of eggs produced by DBM adults developed from infected larvae, and the four subsequent generations at 20 and 25°C, are presented in Figures 1 and 2, respectively. These results show a steadily decreasing number of eggs produced by 1st and 2nd generations of DBM for all concentrations, followed by a dramatic decrease during the 3rd and 4th generations at both temperatures. At 20°C, the number of eggs produced varied significantly ($p < 0.05$) between the control and the treatments for all generations. Similarly, at 25°C, there was a significant ($p < 0.05$) different in the number of eggs produced by parents and subsequent generations;

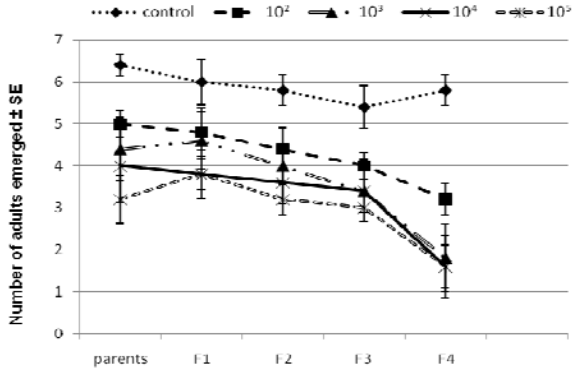

Figure 1. Effect of different concentrations of *Nosema* on the number of adults emerging for parents and 4 subsequent generations at 20°C.

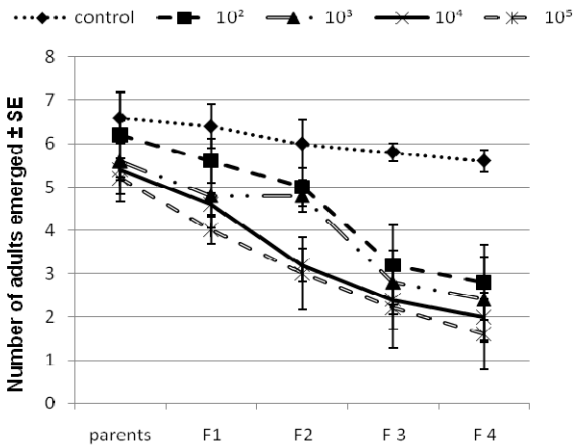

Figure 2. Effect of different concentrations of *Nosema* on the number of adults emerging for parents and 4 subsequent generations at 25°C.

except for eggs produced by the first generation ($p = 0.05$). The fewest ($P = 0.000$) number of eggs were laid when 10^5 spores were used for both temperatures. The decreasing number of eggs may be related to some factors affecting the egg laying process, because of *Nosema* infecting certain tissues (Jolly and Sen, 1972). Reduction of the number of eggs deposited by infected females could be a response to the competition between the host and the microsporidia for nutrients (Goertz et al.2008). In addition, (Diss et al. 1996; Goertz et al.2008) suggested that infected females compensate the loss of nutrients to the microsporidia by producing fewer eggs These observations are consistent with the effects of other *Nosema* spp., such as *N. pyrausta* on the European corn borer, *Ostrinia nubilalis* (Hübner), (Bruck et al., 2001).

The number of adults emerging from infected larvae and 4 subsequent generations were affected by different *Nosema* concentration treated at both 20 and 25°C (Figures 3 & 4 respectively). In general, using a dose of 10^2 and 10^3 gave no significant difference in several generations for both temperatures. However, doses of 10^4 and 10^5 adversely affected adult emergence ($p < 0.05$) for all treatments. The lowest numbers of emergence were recorded at 20°C when 10^5 spores were used. These results are similar with the sub-lethal effects of other microsporidia on insects (Becnel and Andreadis, 1999).

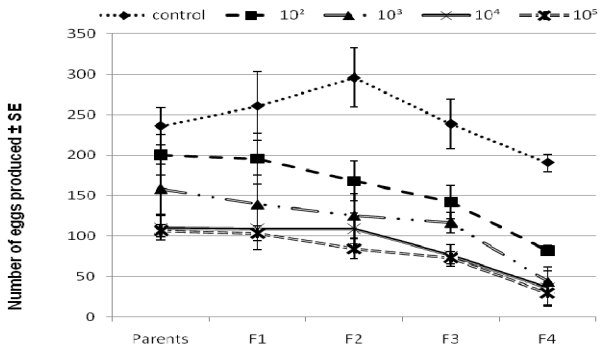

Figure 3. Effect of different concentrations of *Nosema* on the number of eggs produced by parents and 4 subsequent generations at 20°C.

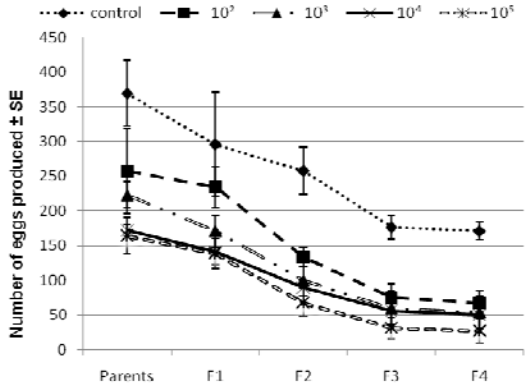

Figure 4. Effect of different concentrations of *Nosema* on the number of eggs produced by parents and 4 subsequent generations at 25°C.

For example, the effectiveness of the microsporidium *Edhazardia aedis* (Kudo) in controlling a semi-natural population of *Aedes aegypti* (L.) was evaluated over a 2-year period, which successfully eliminated the population of *A. aegypti* in Florida (Becnel and Johnson, 2000).

Deformities in infected DBM adults and subsequent generations are also frequently observed in all treatments, even though the number of deformed adults was not significantly different from the control at either 20°C or at 25°C (Figures 5 and 6, respectively). Deformations of adults included wing malformations and reduced adult sizes. Results more similar to ours were observed for spruce budworms, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae), suffering a moderate-heavy infection by *Nosema fumiferanae* (Eveleigh et al., 2007).
Deformations of DBM adults and other lepidoptera could be a result of an ascovirus infection (Furlong and Asgari, 2010) or insecticide treatments, such as methoxyfenozide (Zarate et al., 2011). However, environmental stress throughout development may cause changes in wing shape or body size (Hoffmann et al., 2002).

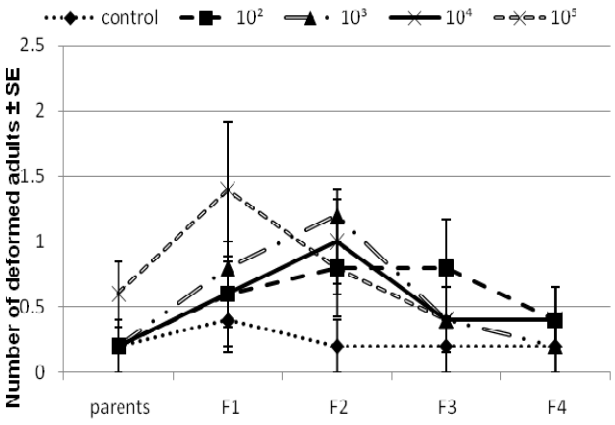

Figure 5. Effect of different concentrations of *Nosema* on the number of abnormal adults emerging for parents and 4 subsequent generations at 20°C.

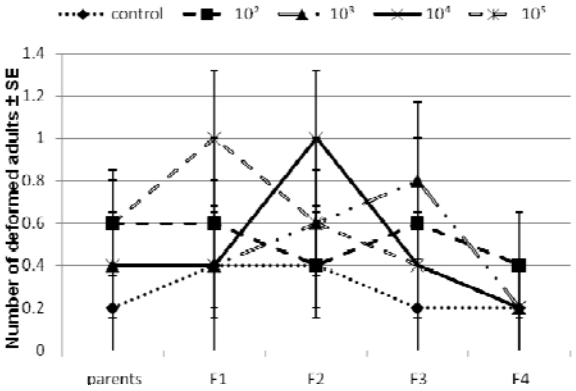

Figure 6. Effect of different concentrations of *Nosema* on the number of abnormal adults emerging for parents and 4 subsequent generations at 25°C.

CONCLUSION

The results confirm that *Nosema* had direct benefits through mortality, as well as the potential for indirect benefits through the reduced number of eggs produced, and for the number of adults emerging for the next generations. This preliminary study should be followed by field experiments to find the best utilization of Nosema for controlling DBM, especially in IBM programs with other controlling agents, such as parasitoids, fungi, viruses, and pesticides.

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Predators in early season brassica crops in South East Queensland (Australia)

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ABSTRACT
Application of insecticides for the control of early season pests in brassica crops can disrupt the endemic natural enemy complex essential for suppression of *Plutella xylostella* (L.) later in the season. A better understanding of the natural enemies present in early season brassicas, and their potential for controlling early season pests is required. Generalist predators are thought to be important in ephemeral cropping systems, responsible for a substantial proportion of pest mortality, but have received comparatively little attention compared with specialist natural enemies. As a first step in clarifying the role of predators in early season plantings, sampling was conducted in a range of crops and locations within the Lockyer Valley (South East Queensland, Australia), with the aim of identifying the most abundant predatory taxa. Visual inspections of plants, sticky traps and pitfall traps were used to monitor predator populations from seedling transplanting until harvest in unsprayed and commercial crops of broccoli, cabbage, cauliflower and Chinese cabbage. Predators included species of Araneae (Theridiidae, Clubionidae, Miturgidae, Lycosidae, Salticidae, Oxyopidae, Araneidae, Tetragnathidae, Thomisidae), Coleoptera (Coccinellidae, Carabidae, Staphylinidae), Diptera (Syrphidae), Neuroptera (Hemerobiidae), Hemiptera (Miridae, Anthocoridae, Lygaeidae, Reduviidae, Nabidae), Dermaptera (Labiduridae) and Hymenoptera (Formicidae). Spiders were the most abundant predators, found consistently at all sampling sites from transplanting onwards. In comparison, populations of predatory insects varied between sampling sites, and foliage-dwelling predatory insects were generally absent from plants for the first two weeks post-transplanting.

Keywords
Predators, spiders, brassicas, early season, integrated pest management

INTRODUCTION
In the Lockyer Valley (South East Queensland), the brassica cropping season extends from February to November. Early season plantings are attacked by *Bemisia tabaci* (Gennadius) biotype B (silverleaf whitefly), *Hellula hydralis* Guenée (centre grub) and *Crocidolomia pavonana* (Fabricius) (cabbage cluster caterpillar). From May to September, *Plutella xylostella* (L.) (diamondback moth) poses the greatest threat to production (Walsh and Furlong 2008). Application of insecticides to control early season pests can disrupt the endemic natural enemy complex, essential for suppression of *P. xylostella* later in the season. A better understanding of the natural enemies present in early season brassicas, and their potential for controlling early season pests is required. This will help inform pest management decisions, reducing the risk of applying unnecessary insecticide treatments.

Generalist predators are thought to be particularly important in ephemeral cropping systems, possessing the attributes required to establish rapidly in recently disturbed habitats at low pest densities (Wiedenmann and Smith 1997; Symondson et al. 2002). There is also growing evidence that predators may be responsible for a substantial proportion of mortality of *P. xylostella* (Furlong et al., 2004a; Furlong et al. 2004b) and *Pieris rapae* (L.), the cabbage white butterfly (Schmaedick and Shelton 1999). Historically, many studies of biological control agents have concentrated on specialist natural enemies, particularly parasitoids (Symondson et al. 2002); the impact of predators on brassica pests has received comparatively little attention (Hosseini et al. 2008). Few surveys of predators in brassica crops in Australia have been performed in Queensland (Heisswolf et al. 1996; Furlong et al. 2004b), New South Wales (Baker 2007) and South Australia (Hosseini et al. 2008). With one exception, predators were not the primary focus of these studies, and consequently limited data were collected. The first step to clarifying the role of natural biological control in early season brassicas is to document the predators present from planting to harvest.

MATERIALS AND METHODS
Sampling was conducted in unsprayed and commercial brassica plantings in the Lockyer Valley. Crops were planted in February, representative of an early season planting in the region.

Unsprayed plantings
In 2009, unsprayed plantings of broccoli (var. Atomic), cabbage (var. Warrior), cauliflower (var. Freemont) and Chinese cabbage (var. Matilda) were established at

Table 1. Commercial brassica planting (2010) site details. Two sites (1 and 2) were sampled at each of two organic farms (ORGA and ORGB) and a conventional farm (CONV).

Farm/site	Pest management strategy	Crop	Insecticides applied	Planting date
ORGA1	Organic (biodynamic)	Broccoli	*Bacillus thuringiensis (Bt)*; azadirachtin (Neem oil)	10/2/2010
ORGA2	Organic (biodynamic)	Cauliflower	*Bt*; azadirachtin (Neem oil)	10/2/2010
ORGB1	Organic	Mixed brassicas	None	22/2/2010
ORGB2	Organic	Broccoli	*Bt*; spinosad (Entrust)	22/2/2010
CONV1	Conventional	Cabbage	None *	15/2/2010
CONV2	Conventional	Cabbage	*Bt*; spinosad (Success); emamectin benzoate (Proclaim); chlorantraniliprole (Coragen)	15/2/2010

* Spray drift onto the site from adjacent crops occurred on at least one occasion

Gatton Research Station (27°32' S, 152°19' E, elevation 98 m). Sampling was conducted over a ten-week period from transplanting (26 February 2009) to harvest (5 May 2009). A randomized complete block design with four replicates was used. Each replicate block (20 m x 50 m) was divided into four plots (9 m x 24 m), each planted with a different brassica type, separated by an unplanted buffer zone (2 m). Plantings were in double rows, with 1.5 m between bed centers, and industry standard spacings between plants within rows. The crop was overhead irrigated as necessary when in-crop rainfall was not sufficient and subjected to standard disease, weed and nutrient management. Two applications of *Bacillus thuringiensis* (*Bt*) (XenTari WG and Dipel DF) were made to reduce large infestations of *C. pavonana*, which would have caused unacceptable damage to the crops if left untreated.

Foliage-dwelling predators were monitored through visual inspections of plants, performed at approximately weekly intervals commencing one week post transplanting. At each inspection, five (8 and 10 week assessments) or ten (remaining assessments) plants of each crop type were selected at random from each replicate. Each plant was examined carefully and all fauna logged. The majority of fauna were identified in the field; however, specimens were collected for subsequent identification where necessary. Adults and immature stages (except eggs) were recorded. At the 4, 8 and 10 week assessments, the selected plants were harvested and placed in sealed bags for examination in the laboratory. For all other weekly assessments, intact plants were examined *in situ*.

Yellow sticky traps (11 cm x 10 cm) were used as an additional sampling technique for foliage-dwelling predators. One trap was placed in the centre of each replicate of each crop type, positioned 40 cm above the ground. Traps were placed in the crop between 30 March and 5 May, examined and replaced weekly.

Pitfall traps, used to sample ground-dwelling predators, were placed in the crop for the duration of the trial. Trap design was based on that of Furlong et al. (2004b): each trap consisted of a 275 ml plastic cup (7.5 cm diameter), placed within a second larger cup (320 ml), buried with the rim level with the soil surface. Traps were half-filled with a weak detergent solution and covered with a plastic disc (18 cm diameter), supported approximately 3 cm above the soil surface by three nails. Three pitfall traps were placed in each replicate of each brassica type, arranged along a diagonal across the plot. Traps were examined and replaced weekly.

Cumulative total predator numbers from planting to harvest in each brassica type were compared using analysis of variance followed by Fisher's least significant difference (LSD) tests to distinguish between the means (GenStat 11th edition, VSN International Ltd). All life stages except eggs were included in the analyses.

Commercial plantings

In 2010, sampling was carried out in commercial brassica plantings at three vegetable farms in the Lockyer Valley region, situated within 20 km of Gatton Research Station. Crops were planted 10 - 22 February 2010. Two of the farms were certified organic (ORGA and ORGB); the third practiced conventional pest management (CONV), by far the most prevalent system operating in the Lockyer Valley. Two sites were sampled at each of the three farms (Table 1). In order to obtain information on effects of pesticides on natural enemy populations, one site at each of the ORGB and CONV farms was set aside and no pesticides were applied (ORGB1 and CONV1). Crops at the second site at each of these farms (ORGB2 and CONV2) were managed according to the growers' usual practices.

Visual inspections of plants were carried out approximately weekly, commencing one week post transplanting and finishing seven (ORGB), eight (CONV) or nine (ORGA) weeks later. Thirty plants were inspected at each site, following the procedure described for the unsprayed plantings. Five pitfall traps and five yellow sticky traps were placed at each site and replaced approximately twice weekly for the duration of the trial.

As the trial in commercial plantings was not replicated, averages were calculated from the subsamples (30 plants or 5 traps) collected at each site. All life stages except eggs were included in this calculation.

Table 2. Predators encountered during sampling from foliage, pitfall traps and yellow sticky traps in unsprayed and commercial plantings

Order	Family	Species
Araneae	Theridiidae *	*Cryptachaea veruculata* (Urquhart)
	Clubionidae *	*Clubiona* sp.
	Miturgidae *	*Cheiracanthium* sp.
	Lycosidae *	*Artoria* sp.
	Salticidae, Oxyopidae, Araneidae, Tetragnathidae, Thomisidae	Unidentified
Coleoptera	Coccinellidae	*Coccinella transversalis* Fabricius; *Coelophora inaequalis* (Fabricius); *Diomus notescens* (Blackburn); *Hippodamia variegata* (Goeze); *Harmonia conformis* (Boisduval); *H. octomaculata* (Fabricius); *Stethorus* sp.
	Carabidae	Unidentified
	Staphylinidae	Unidentified
	Melyridae	*Dicranolaius bellulus* (Guérin-Méneville)
	Cantharidae	*Chauliognathus lugubris* (Fabricius)
Diptera	Syrphidae	Unidentified
Neuroptera	Hemerobiidae	*Micromus* sp.
Hemiptera	Miridae	*Taylorilygus* sp.; *Deraeocoris signatus* (Distant); *Campylomma liebknechti* (Girault)
	Reduviidae	*Pristhesancus* sp.
	Anthocoridae	*Orius* sp.
	Lygaeidae	*Geocoris* sp.
	Nabidae	*Nabis kinbergii* Reuter
	Pentatomidae	Unidentified
Dermaptera	Labiduridae	*Labidura truncata* Kirby
Hymenoptera	Formicidae	Unidentified
Thysanoptera	Aeolothripidae	Unidentified
Mantodea	Mantidae	Unidentified
Class Chilopoda		Unidentified

* Specimens of commonly occurring spiders (Theridiidae, Clubionidae, Miturgidae and Lycosidae) were sent to Owen Seeman (Queensland Museum, Brisbane, QLD) for expert identification; other, unidentified species also occurred.

RESULTS AND DISCUSSION

The range of predatory arthropods encountered (Table 2) was similar to that recorded in previous sampling studies conducted in the Lockyer Valley (Heisswolf et al. 1996; Furlong et al. 2004b).

Foliage-dwelling predators

Foliage-dwelling predators most commonly observed during visual inspections of plants were Araneae (predominantly Theridiidae, Clubionidae and Miturgidae), Coccinellidae, Hemerobiidae, Syrphidae and Hemiptera (predominantly Miridae and Anthocoridae). Araneae were the most numerous predators at the majority of sites. In comparison, relative abundance of the predatory insect taxa differed between crops and sampling sites (Tables 3 and 4). Spiders are the most abundant predators in Australian agroecosystems (Whitehouse and Lawrence 2001). Experimental manipulation of spider populations has demonstrated their impact on pests in a variety of cropping systems (Sunderland 1999), including vegetables (Riechert and Bishop 1990).

A greater number of predators were encountered in Chinese cabbage than any other brassica type ($F = 49.42$, $P < 0.001$), probably due to the presence of large numbers of aphids. The aphidophagous coccinellids were the dominant predator in this crop.

Predatory insects such as coccinellids and syrphids were not observed in crops until at least 18 days post transplanting (Figures 1 and 2). Prasad et al. (2009), monitoring aphids and their natural enemies in brassicas in British Columbia (Canada), observed a lag of several weeks in colonization by syrphids and other aphid predators. These authors concluded that syrphids act on

aphids in a density-dependent manner, and hence they are often unable to control populations before damage exceeds threshold levels.

Whereas predatory insects were absent from newly transplanted seedlings, spiders were present from the first assessment at five days post transplanting. Aerial immigration via 'ballooning' enables these predators to colonize newly cultivated fields much more quickly than many other predators (Bishop and Riechert 1990).

With the exception of Chinese cabbage (data from this crop excluded from Figure 1), spiders outnumbered the total combined predatory insects for the duration of the crop in unsprayed plantings (Figure 1). Similarly, in commercial plantings, predatory insects did not achieve numbers comparable with spiders until crops were close to harvest (Figure 2).

Sampling with sticky traps resulted in a similar range of predatory insects as visual inspections, but in different relative abundance (Tables 5 and 6). As sticky traps caught predominantly adults, they did not reflect the increase in predator populations revealed through visual sampling. Furthermore, whereas visual sampling found no predatory insects on foliage until 18 days post transplanting, adults were caught on sticky traps during this period. These results highlight the importance of using several sampling techniques, corroborating the findings from previous sampling studies (Schmaedick et al. 1997; Hosseini et al. 2008).

Table 3. Commonly observed predators in unsprayed plantings (visual inspections of plants)

Brassica type	Araneae	Coleoptera (Coccinellidae)	Neuroptera (Hemerobiidae)	Diptera (Syrphidae)	Hemiptera	Total predators
Broccoli	6.18 ± 1.01 (79.17%)	0.20 ± 0.08 (2.56%)	0.58 ± 0.21 (7.37%)	0.53 ± 0.18 (6.73%)	0.33 ± 0.16 (4.17%)	7.80 ± 1.08 a
Cabbage	5.78 ± 0.81 (79.93%)	0.55 ± 0.25 (7.61%)	0.13 ± 0.09 (1.73%)	0.30 ± 0.30 (4.15%)	0.48 ± 0.17 (6.57%)	7.23 ± 1.19 a
Cauliflower	11.70 ± 2.88 (83.72%)	0.28 ± 0.15 (1.97%)	0.45 ± 0.21 (3.22%)	0.83 ± 0.45 (5.90%)	0.73 ± 0.06 (5.19%)	13.98 ± 3.38 a
Chinese cabbage	12.23 ± 0.77 (17.72%)	32.75 ± 4.16 (47.48%)	5.48 ± 1.69 (7.94%)	12.93 ± 4.14 (18.74%)	5.60 ± 0.83 (8.12%)	68.98 ± 7.35 b

Values are cumulative means ± standard errors per plant (n = 4 replicate blocks, 5 or 10 plants sampled per block)

Figures in parentheses are relative abundance expressed as a percentage of the total predators per plant

Total predator means followed by different letters differ significantly (LSD, $P < 0.05$)

Table 4. Commonly observed predators in commercial plantings (visual inspections of plants)

Sampling site	Araneae	Coleoptera (Coccinellidae)	Neuroptera (Hemerobiidae)	Diptera (Syrphidae)	Hemiptera	Total predators
ORGA1	3.11 (74.34%)	0.22 (5.31%)	0.15 (3.54%)	0.63 (15.04%)	0.07 (1.77%)	4.19
ORGA2	3.30 (63.12%)	0.37 (7.09%)	0.30 (5.67%)	1.04 (19.86%)	0.22 (4.26%)	5.22
ORGB1	0.81 (29.82%)	1.19 (43.86%)	0.05 (1.75%)	0.57 (21.05%)	0.10 (3.51%)	2.71
ORGB2	1.86 (78.00%)	0.19 (8.00%)	0.00 (0.00%)	0.24 (10.00%)	0.10 (4.00%)	2.38
CONV1	1.75 (82.35%)	0.13 (5.88%)	0.04 (1.96%)	0.04 (1.96%)	0.17 (7.84%)	2.13
CONV2	1.88 (93.75%)	0.04 (2.08%)	0.04 (2.08%)	0.00 (0.00%)	0.04 (2.08%)	2.00

Values are cumulative means per plant (30 plants sampled per site)

Figures in parentheses are relative abundance expressed as a percentage of the total predators per plant

Table 5. Sticky trap captures for commonly caught predators in unsprayed plantings

Brassica type	Araneae	Coleoptera (Coccinellidae)	Neuroptera (Hemerobiidae)	Diptera (Syrphidae)	Hemiptera	Total predators
Broccoli	0.95 ± 0.05 (9.09%)	2.90 ± 1.38 (27.75%)	0.70 ± 0.24 (6.70%)	0.65 ± 0.39 (6.22%)	5.25 ± 3.35 (50.24%)	10.45 ± 3.10 a
Cabbage	2.90 ± 0.95 (25.33%)	5.80 ± 1.68 (50.66%)	1.00 ± 0.41 (8.73%)	0.60 ± 0.60 (5.24%)	1.15 ± 0.43 (10.04%)	11.45 ± 1.23 a
Cauliflower	0.75 ± 0.48 (5.08%)	3.75 ± 1.03 (25.42%)	2.25 ± 0.75 (15.25%)	0.50 ± 0.29 (3.39%)	7.50 ± 1.44 (50.85%)	14.75 ± 1.65 a
Chinese cabbage	1.25 ± 0.95 (2.50%)	32.50 ± 6.89 (65.00%)	2.75 ± 1.80 (5.50%)	0.75 ± 0.25 (1.50%)	12.75 ± 2.50 (25.50%)	50.00 ± 7.36 b

Values are cumulative means ± standard errors per trap (n = 4 replicate blocks, 1 trap per block)
Figures in parentheses are relative abundance expressed as a percentage of the total predators per trap
Total predator means followed by different letters differ significantly (LSD, $P < 0.05$)

Table 6. Sticky trap captures for commonly caught predators in commercial plantings

Sampling site	Araneae	Coleoptera (Coccinellidae)	Neuroptera (Hemerobiidae)	Diptera (Syrphidae)	Hemiptera	Total predators
ORGA1	4.77 (45.93%)	3.15 (30.37%)	0.08 (0.74%)	1.54 (14.81%)	0.85 (8.15%)	10.38
ORGA2	8.63 (80.99%)	1.09 (10.23%)	0.00 (0.00%)	0.78 (7.34%)	0.15 (1.44%)	10.65
ORGB1	11.56 (77.61%)	0.89 (5.97%)	0.00 (0.00%)	1.78 (11.94%)	0.67 (4.48%)	14.89
ORGB2	17.33 (87.64%)	0.67 (3.37%)	0.00 (0.00%)	1.33 (6.74%)	0.44 (2.25%)	19.78
CONV1	7.83 (45.63%)	1.33 (7.77%)	0.33 (1.94%)	6.67 (38.83%)	1.00 (5.83%)	17.17
CONV2	12.33 (65.49%)	1.83 (9.73%)	0.50 (2.65%)	3.33 (17.70%)	0.83 (4.42%)	18.83

Values are cumulative means per trap (5 traps per site)
Figures in parentheses are relative abundance expressed as a percentage of the total predators per trap

Ground-dwelling predators

Commonly trapped ground-dwelling predators were Lycosidae, Labiduridae, Carabidae and Formicidae (Tables 7 and 8). Small numbers of Staphylinidae, Chilopoda and unidentified Araneae were also encountered.

Although Formicidae formed a large proportion of trap catch, activity was highly localized; large catches often occurred in a single trap at each site. Therefore, although results for Formicidae are presented, they were excluded from the calculation of relative abundance. The contribution of ants to biological control is uncertain: although they prey on a variety of pest species, they may also have a negative impact on some beneficial species, and can actively protect honeydew-producing hemipteran pests from natural enemies (Chong et al. 2010).

Previous sampling studies in Australian brassica crops found Lycosidae to be the most abundant predator in pitfall catches (Furlong et al. 2004b; Hosseini et al. 2008). This was not so in the current study, although lycosids were trapped consistently at all sites (Tables 7 and 8) and all sampling dates (Figures 3 and 4). In comparison, catches of the predatory insects (Labiduridae, Carabidae and Formicidae) varied considerably between sites and sampling dates.

Table 7. Pitfall trap captures for commonly caught predators in unsprayed plantings

Brassica type	Araneae (Lycosidae)	Dermaptera (Labiduridae)	Coleoptera (Carabidae)	Total predators*	Hymenoptera (Formicidae)
Broccoli	6.08 ± 1.15 (27.34%)	15.42 ± 6.17 (69.29%)	0.75 ± 0.44 (3.37%)	22.25 ± 6.26 ab	11.75 ± 3.83
Cabbage	8.00 ± 1.96 (30.77%)	17.50 ± 5.82 (67.31%)	0.50 ± 0.10 (1.92%)	26.00 ± 3.89 b	28.83 ±15.23
Cauliflower	8.38 ± 1.69 (26.27%)	22.54 ± 10.66 (70.72%)	0.96 ± 0.29 (3.01%)	31.88 ± 10.31 b	8.46 ± 3.04
Chinese cabbage	6.58 ± 1.13 (46.20%)	6.83 ± 2.76 (47.95%)	0.83 ± 0.29 (5.85%)	14.25 ± 3.49 a	12.33 ± 3.24

Values are cumulative means ± standard errors per trap (n = 4 replicate blocks, 3 traps per block)

Figures in parentheses are relative abundance expressed as a percentage of the total predators (excluding Formicidae*) per trap

Total predator means followed by different letters differ significantly (LSD, $P < 0.05$)

Table 8. Pitfall trap captures for commonly caught predators in commercial plantings

Sampling site	Araneae (Lycosidae)	Dermaptera (Labiduridae)	Coleoptera (Carabidae)	Total predators*	Hymenoptera (Formicidae)
ORGA1	3.73 (71.79%)	0.27 (5.13%)	1.20 (23.08%)	5.20	72.13
ORGA2	4.05 (24.24%)	7.64 (45.72%)	5.02 (30.04%)	16.70	33.55
ORGB1	3.56 (32.79%)	0.00 (0.00%)	7.29 (67.21%)	10.84	53.87
ORGB2	4.71 (78.52%)	0.20 (3.33%)	1.09 (18.15%)	6.00	1.80
CONV1	6.85 (88.98%)	0.13 (1.73%)	0.72 (9.29%)	7.70	16.73
CONV2	6.46 (90.49%)	0.40 (5.60%)	0.28 (3.90%)	7.14	15.55

Values are cumulative means per trap (5 traps per site)

Figures in parentheses are relative abundance expressed as a percentage of the total predators (excluding Formicidae*) per trap

Effects of pest management practice on the predator complex in commercial plantings

As the trial in commercial plantings was not replicated, results should be interpreted with caution. However, there was some indication of an effect of pest management practice on foliage-dwelling predators. The total number of predators was highest at the two ORGA sites (an organic farm practicing biodynamics) and lowest at the site exposed to conventional pesticide applications (CONV2) (Table 4). Several of the predatory insect taxa were more abundant at sites where pesticides were withheld (ORGB1 and CONV1) than the corresponding sites exposed to pesticides (ORGB2 and CONV2). Observations of pest populations suggest that these differences in predator abundance may have been linked to numbers of aphids, and not solely a direct effect of pesticides, with organic growers more likely to tolerate an aphid infestation.

Effects of pest management practice on the ground dwelling predators were less apparent. There were more carabid beetles in sites from which pesticides were withheld (ORGB1 and CONV1) than the sites exposed to pesticides (ORGB2 and CONV2), but no other consistent differences between sites (Table 8) were observed.

CONCLUSION

Sampling over a two-year period has documented arthropod predators in early season brassica crops in South East Queensland, and provided an indication of the relative abundance of the most commonly observed taxa. Araneae were generally more numerous than predatory insects, and were found consistently at all sampling sites from transplanting to harvest. There was some indication of an effect of pest management practice on foliage-

dwelling predators, although it is likely that differences in predator abundance were linked to differing aphid populations, rather than a direct effect of pesticides.

The dominance of spiders, particularly in newly transplanted seedlings, suggests their potential contribution to control of pests in short-term crops should not be ignored. However, an abundance of a particular predator does not necessarily signify that it will have an impact on pest species. Work is ongoing to investigate predation by some of the more commonly found spiders on key brassica pests.

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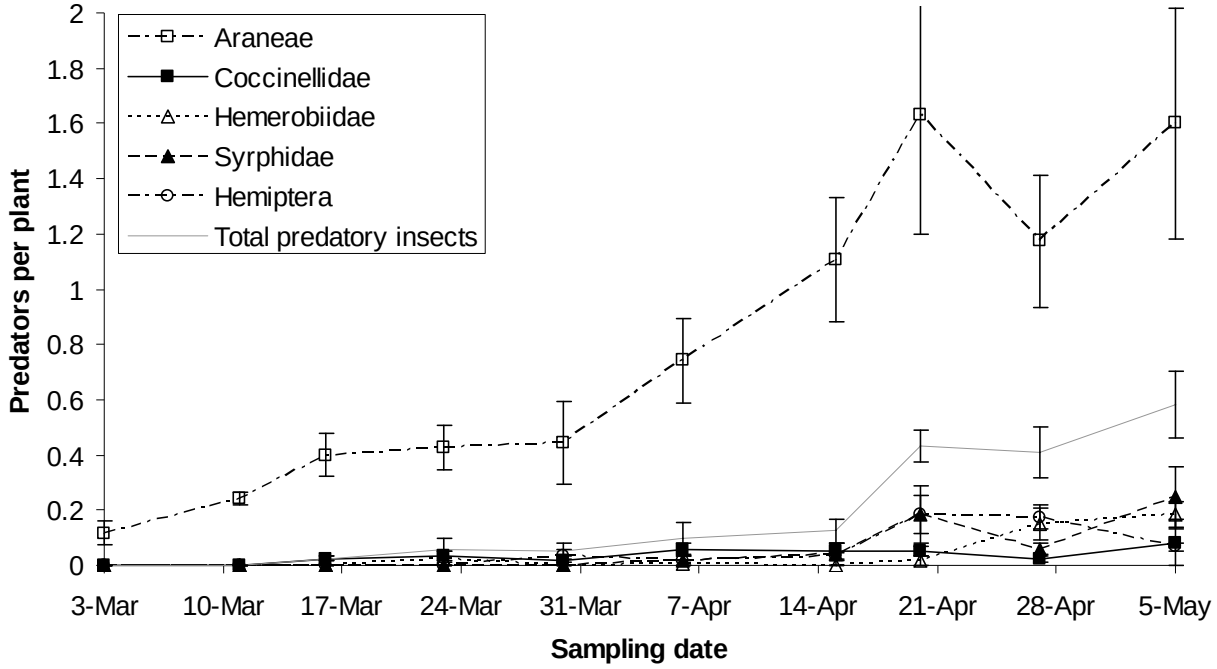

Figure 1. Commonly observed predators by date in unsprayed plantings (visual inspections of plants). Values are means ± standard errors per plant (n = 4 replicate blocks, 15 or 30 plants sampled per block from three crops, Chinese cabbage excluded)

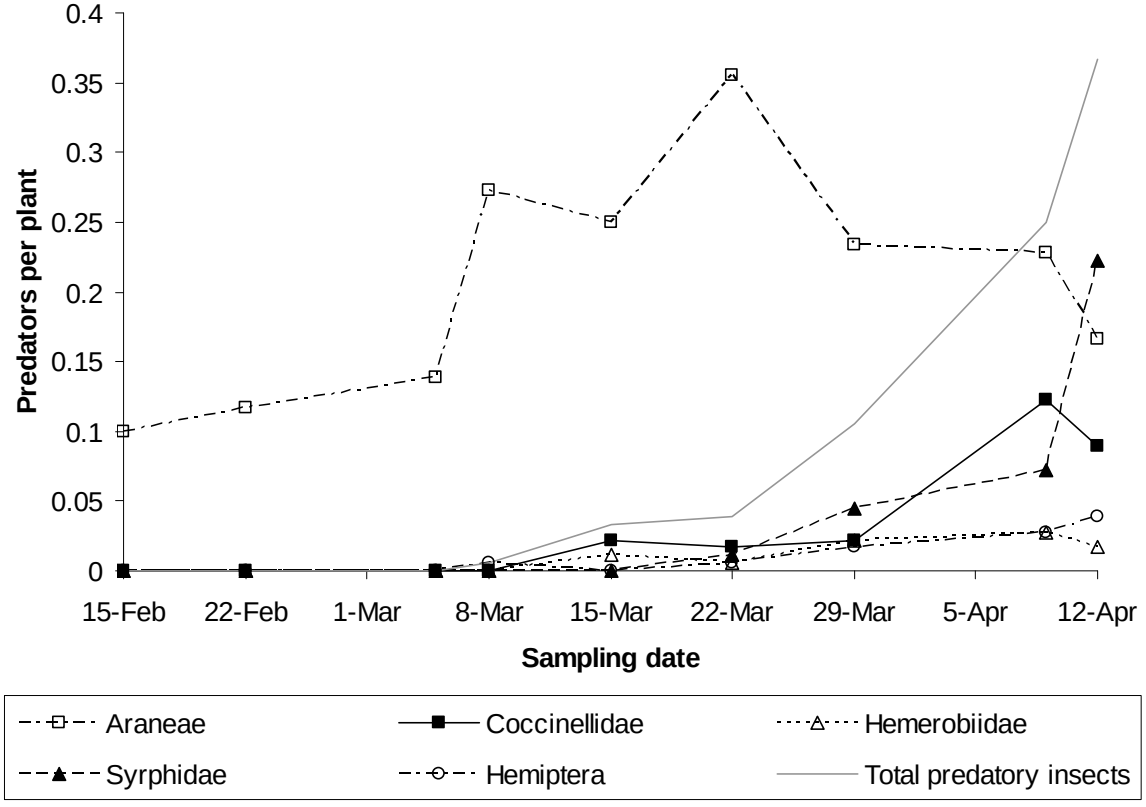

Figure 2. Commonly observed predators by date in commercial plantings (visual inspections of plants). Values are means per plant (180 plants sampled from six sites)

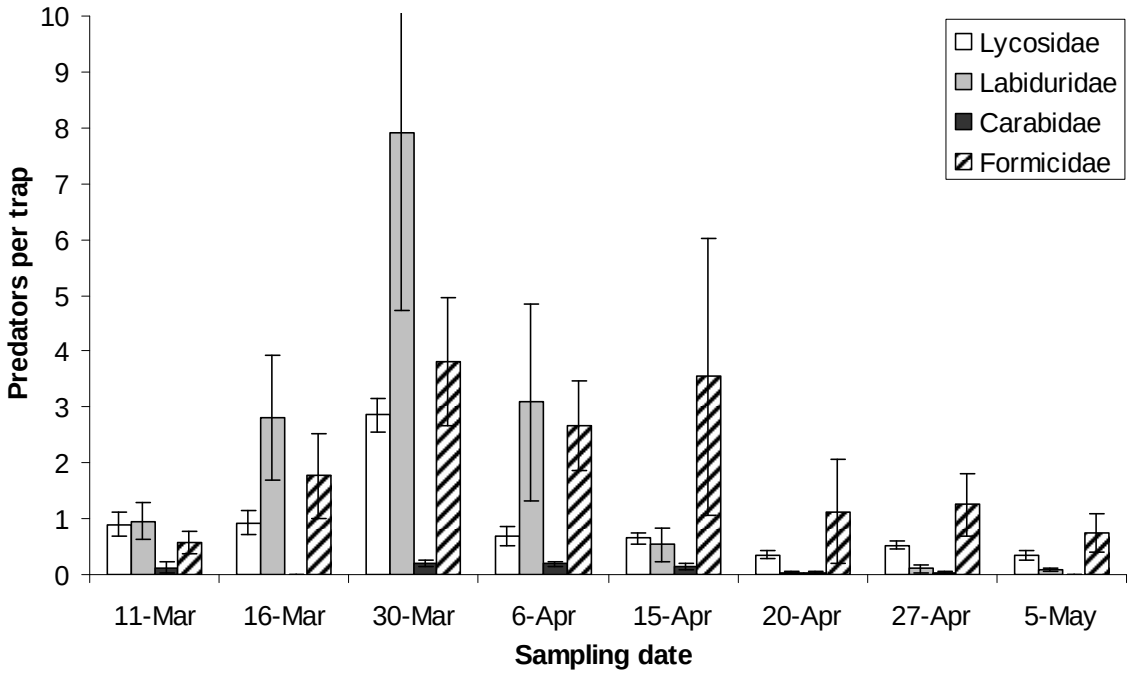

Figure 3. Pitfall trap captures by date for commonly caught predators in unsprayed plantings. Values are means ± standard errors per trap (n = 4 replicate blocks, 12 traps per block in four crops)

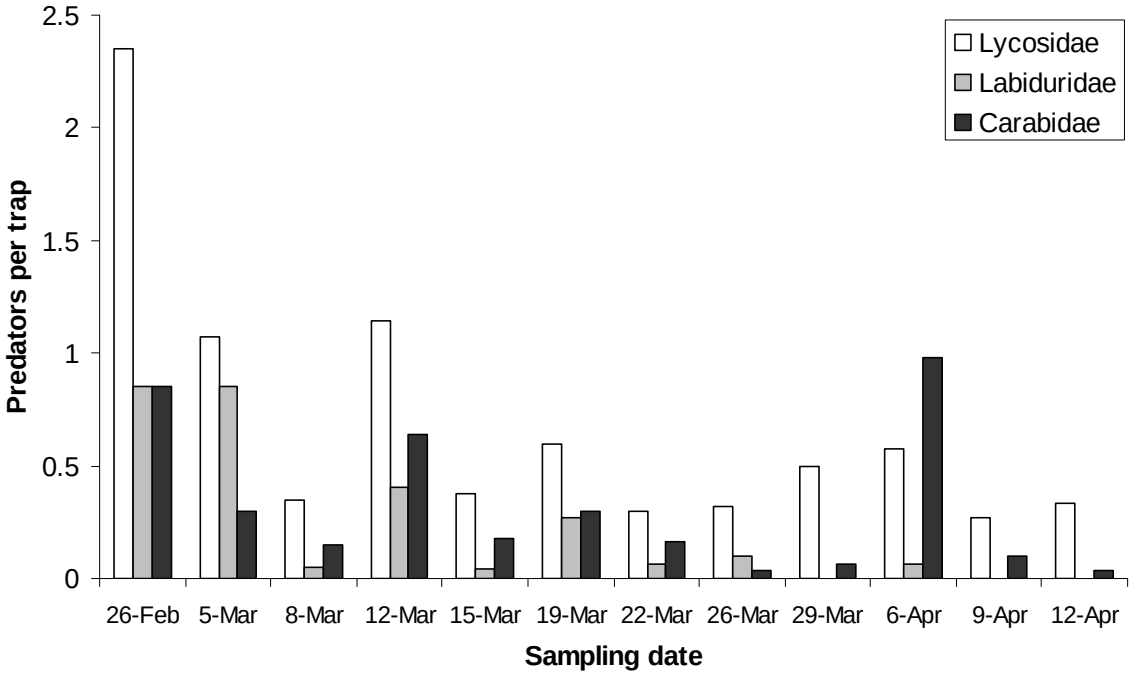

Figure 4. Pitfall trap captures for commonly caught predators in commercial plantings. Values are means per trap (30 traps at six sites). Average catches of Formicidae greatly exceeded other predators at all dates: between 0.7 (12 April) and 14.3 (26 February)

Mode of action and efficacy of Bioact-T EC6K and ETOGROWTH TM– EC (612) against diamondback moth, *Plutella xylostella* (Lin.)

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ABSTRACT
The action modes and efficacy of two botanical insecticides, Bioact and ETOGROWTH were tested against *Plutella xylostella*. Both compounds possessed contact toxicity, antifeedant activities and repellency effect. ETOGROWTH possessed 2.7 times less contact toxicity to DBM larvae than that of cypermethrin but was 3.7 times stronger than that of Bioact at 2.5 ml/l of respective compounds. Bioact exhibited significant deterrent activities at 0.03125 ml/l and above, with exceptional of 0.0625 ml/l. The FDI value was more than 43 at 0.03125 ml/l. Negative values of RGR and FDI were observed at 0.5 ml/l of Bioact. Similarly, ETOGROWTH showed nutritional and feeding deterrent activities against DBM larvae at 0.0625 ml/l and above, with the FDI value above 50. Bioact and ETOGROWTH produced repellent index of class III or above to DBM larvae. Hence, Bioact and ETOGROWTH were more useful to control the DBM larvae as semiochemicals other than poison compounds.

Keywords
Botanical insecticide, mode of action, efficacy, Diamondback moth, semiochemicals

INTRODUCTION
As people become rich and the lifestyle is improving, the demand for organic food and organic agro-products is increasing. Organic produce is just as important for the societal benefits it brings as it is for the quality or health benefits of the food (LOTTER 2003, Oecd. 2003, Alex 2006). To produce high quality and organic products, the use of the natural pesticides bio-agents for integrated pest management system on crops and vegetables become essential for farmers, especially the organic farmers. In this report, two botanical insecticides, namely bioact-t ec6k and etogrowth^{tm} – ec (612) had been tested on the mode of action and evaluated on the insecticidal properties against diamondback moth, *p. xylostella*.

MATERIALS AND METHODS

Test insects
DBM larvae were collected from Singapore farms in 1989 and were maintained in insectary room, Plant Health Centre, Agri-Food and Veterninary Authority of Singapore, 6 Perahu Road, Singapore 718827. Fresh leaves of Gailan, *Brassica* sp., free from insecticides were used for rearing the DBM larvae in the insect cages. Small pots growing with Gailan for about 20 days were put in the insect cages. DBM pupae were introduced into the cages. The hatched DBM larvae were rearing on the gailan. The larvae will be transferred to a new plant should the leaves be eaten up. DBM pupae were collected from the plants in the cages for population maintanece.

3 instar DBM were used for the experiments. Fresh Gailan leaves free from insecticides punctured with 9 cm Ø puncture. 100 g of the leaf disc was used for each treatment.

Insecticides
Bioact-T EC6K, a compound containing 40% ingredients from *Azadirachta indica* blended with adjuvant of some other botanical extracts and ETOGROWTH^{TM} – EC (612), 12% of Etofenprox formulated in proprietary blends of botanical extracts, were tested. Cypermethrin 2.8% EC was used as a reference insecticide.

Contact toxicity test
0.9 cm diameter leaf discs were punched out from fresh gailan leaves and soaked in various concentrations for ten seconds. The treated leaf discs were dried for 1 hr and then placed in the glass-dishes. Five larvae of *P. xylostella* were introduced into each arena (Φ 9 cm, height 1.4 cm glass-dish) containing three treated discs. The concentrations tested were 0.625 ml/l, 1.25 ml/l, 2.5 ml/l, 5 ml/l and 20 ml/l. Controls were treated with carrier solution alone. Three replicates were made for each treatment. After a 24, 48 and 72-hour exposure time, the insects were examined for mortality. Mortality was calculated using Abbot's formula. At the same time, LC_{50} and relative toxic indices were also calculated..

Mortality was calculated using Abbott's formula (Abbott 1987). LC_{50} and relative toxic indices were also calculated

Mortality (%) = [(percent test mortality – percent control mortality) / (100 - percent control mortality)]*100

Relative toxic index (RTI) = $(M_t / M_C) * 100$ *M_C: caused by control insecticide at 2.5ml/l*
M_{t5}: caused by test insecticide at 5ml/l

Antifeedant action

A no-choice leaf disk bioassay was employed. The antifeedant parameters including relative growth rate (RGR), relative consumption rate (RCR) and the efficiency of conversion of ingested food (ECI) of *P. xylostella*, were calculated. For the feeding deterrent index (FDI), formula described by Isman *et al.* (1990) was adopted.

The following formulas were used for calculating the nutritional indices:

RGR = (A-B) / (B × day) ----------I

where A = weight of live insects on the third day (mg) / number of live insects on the third day, B = original weight of insects (mg) / original number of insects.

RCR = D / (B × day) ----------II

where D = weight ingested (mg) / number of live insects on the third day.

ECI (%) = (RGR / RCR) × 100 ----------III

FDI (%) = [(C-T) / C] × 100 ----------IV

where C = the consumption of control disks and T = the consumption of treated disks, as the control and treated disks were placed in separate vials.

Repellent activities

Repellent test for Bioact-T EC6K and ETOGROWTH™ – EC (612) was adopted from the standard method (No.3) of McDonald *et al.* (1970) with some modifications (Talukder and Howse, 1993, 1994, 1995).

The average of the counts was converted to percentage repellency (PR) as follows:

PR = 2 * (C - 50)

where C was the percentage of insects on the untreated half filter paper disk.

Positive values (+) expressed repellency and negative values (-) expressed attraction. The averages were then categorised according to the following scale:

Percent repellency (%)	Class
> 0.01 to < 0.1	0
0.1 - 20	I
20.1-40	II
40.1-60	III
60.1-80	IV
80.1-100	V

Data (PR) were analyzed using analysis of variance (ANOVA) (two-way) and Duncan's multiple range test after transforming them into *arc sine* √ percentage values.

Data analyses

The data from the various tests were analyzed using statistical software packages such as Probit Analysis and MULSTAT.

RESULTS AND DISCUSSION

Bioact and ETOGROWTH had moderate contact toxicity against the larvae of DBM. ETOGROWTH, with a LC_{50} value of 1.045 possessed stronger toxicity to DBM than that of Bioact, which recorded a LC_{50} value of 1.689 (please see table 1 attached at the end of this paper.

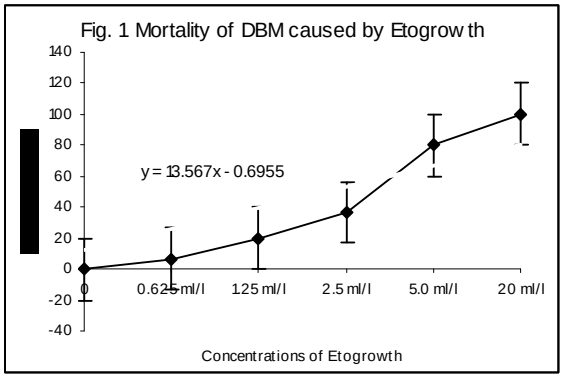

Figure 1. Mortality of DBM caused by Etogrowth

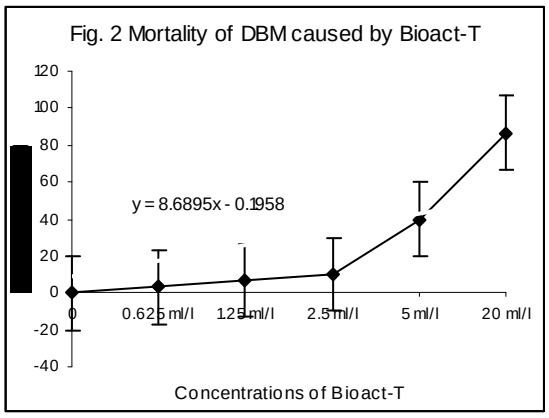

Figure 2. Mortality of DBM caused by Bioact-T

ETOGROWTH at a concentration of 2.5 ml/l caused 36.67% mortality of DBM while at the same concentration Bioact gave only 10.00% mortality of

DBM, which was 3.8 times lower than that of the former (Fig. 1 and 2).

Table 1. Relative toxic index of Bioact-T EC6K and ETOGROWTHTM – EC (612) to Cypermethrin*

Chemical	Concentration	Mortality	Relative toxic index
Cypermethrin	2.5 ml/l	100.00±0.00	1.00
Etogrowth**	0.625 ml/l	6.67±0.58	0.07
	1.25 ml/l	20.00±0.00	0.20
	2.5 ml/l	36.67±0.58	0.38
	5 ml/l	80.00±1.00	0.80
	20 ml/l	100.00±0.00	1.00
Bioact-T	0.625 ml/l	3.30±0.58	0.03
	1.25 ml/l	6.67±0.58	0.07
	2.5 ml/l	10.00±1.00	0.10
	5 ml/l	40.00±1.00	0.40
	20 ml/l	86.67±0.58	0.87

* The values were based on five concentrations, three replicates of 10 insects each.
** Bioact-T = Bioact-T EC6K; Etogrowth = ETOGROWTH^{TM} – EC (612).

RTI values showed that ETOGROWTH possessed 2.7 times less contact toxicity to DBM larvae than that of cypermethrin, but was 3.7 times stronger than that of Bioact at 2.5 ml/l of respective compounds (Table 1 and Fig. 3).

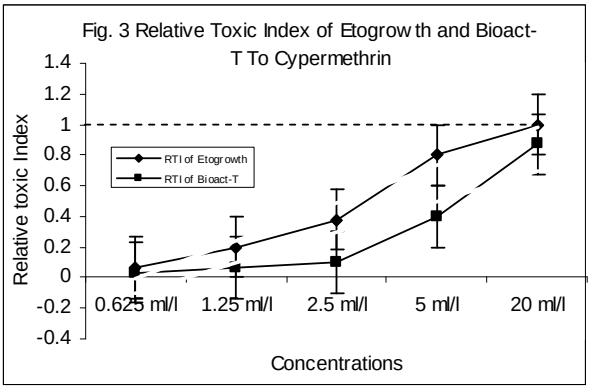

Figure 3. relative Toxic Index of Etogrowth and Bioact-T to Cypermethrin

RGR was significantly reduced for DBM larvae fed on the leaf discs treated with ETOGROWTH. As the concentrations increased, the RGR was reduced further. Nevertheless, RCR and ECI of DBM were decreased as the concentration of ETOGROWTH increased. At the concentration of 0.5 ml/l, negative RCR and ECI were observed (Fig. 4).

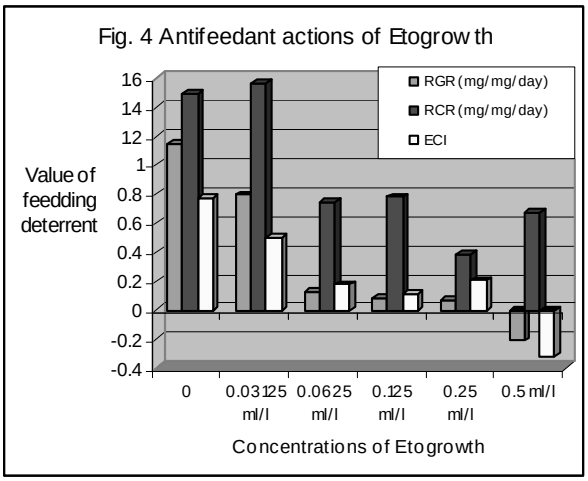

Figure 4. Antifeedant actions of Etogrowth

A similar trend of RGR, RCR and ECI of DBM were observed when the insect was fed on the leaf discs treated with various concentrations of Bioact (Fig. 5).

ETOGROWTH had its highest antifeedant effect at a concentration of 0.25 ml/l with FDI value of 0.742 while that of Bioact was at 0.5 ml/l with FDI value of 0.86. Interestingly, for food treated with 0.031 ml/l of Bioact and ETOGROWTH and 0.063 ml/l of Bioact, feeding stimulation effects were recorded (Fig. 6).

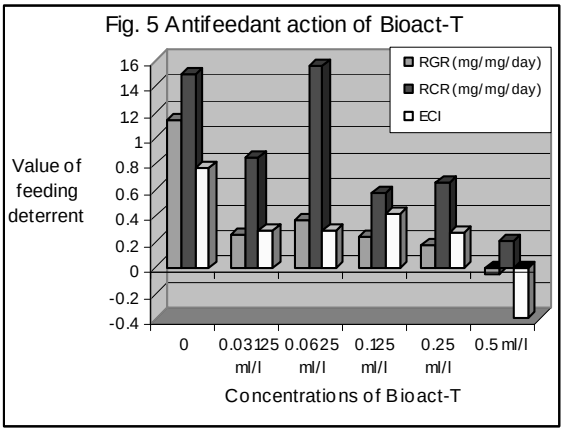

Figure 5. Antifeedant action of Bioact-T

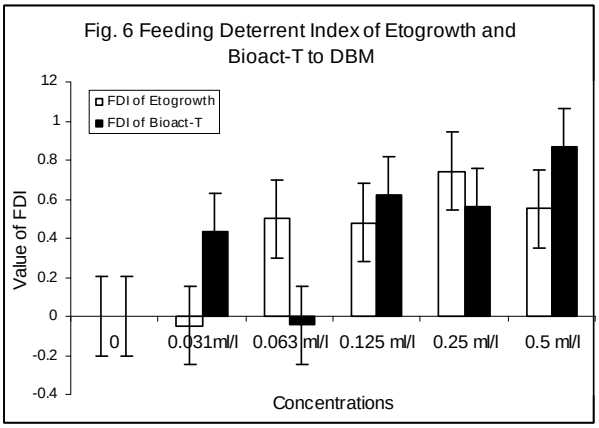

Figure 6. Feeding Deterrent Index of Etogrowth and Bioact-T to DBM

DBM fed on the leaf discs treated with Bioact and ETOGROWTH, as the concentrations increased, the RGR, RCR and ECI of DBM were decreased accordingly. Both less food consumption and lower efficiency of food utilization contributed to the growth inhibition (Rosenthal, 1988). The phenomena meant that though the insects could take some treated food, they could hardly utilise it as nutrition for their body building and their population development. To maintain their life, they had to use their stored energy which led to the negative value of RGR (Berenbaum, 1992; Rosenthal, 1991). The results confirmed an earlier hypothesis that the mechanism of growth disruption by azadirachtin was separate from that associated with feeding inhibition (Koul *et al.*,1985).

DBM were repelled constantly by Bioact and ETOGROWTH at all times (Fig. 7 & 8). At 4 h exposure, the repellent effects of both the compounds, Bioact and ETOGROWTH to DBM reached to their highest level, class IV.

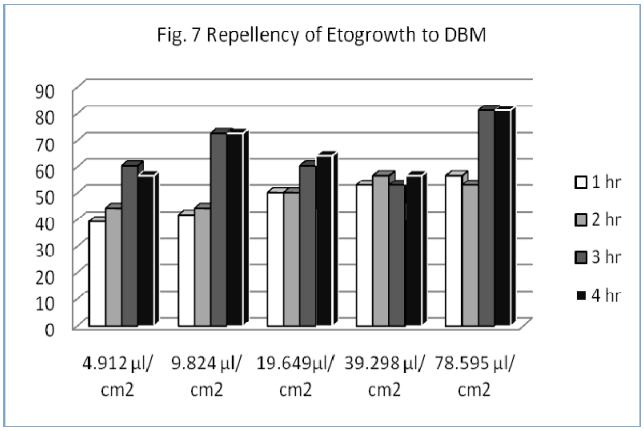

Figure 7. repellency of Etogrowth to DBM

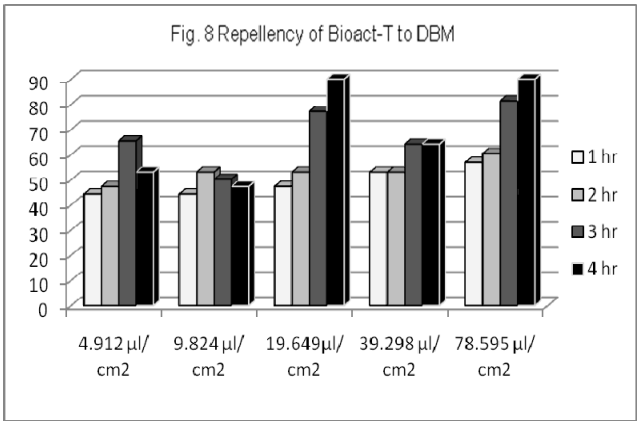

Figure 8. Repellency of Bioact-T to DBM

Bioact and ETOGROWTH exhibited to be strongly repellent to DBM at all concentrations tested (Fig. 9). At concentration of 78.595 µl/cm^2, the repellent effects of both Bioact and ETOGROWTH to DBM reached to class IV.

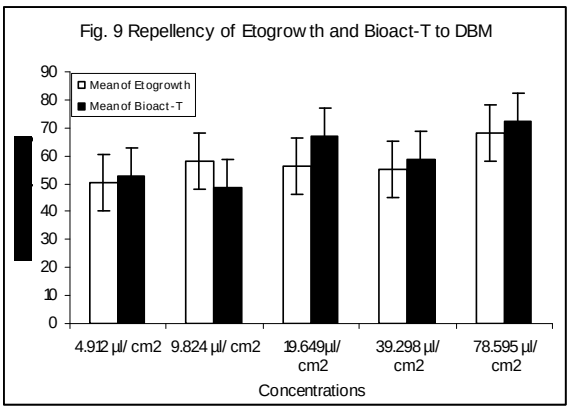

Figure 9. Repellency of Etogrowth and Bioact to DBM

Although the value of repellent class of Bioact was slightly higher than that of ETOGROWTH, the difference was not significant.

CONCLUSION

The results of the experiments showed that Bioact and ETOGROWTH had less potential in killing *P. xylostella* larvae than that of Cypermethrin. The RTI values revealed that ETOGROWTH possessed higher toxicity to *P. xylostella* larvae than that of Bioact.

Both Bioact and ETOGROWTH, on the other hand, presented fairly strong antifeedant property to DBM. Those feeding deterrent activities were not only in RGR and RCR, but in ECI as well, at concentrations of 0.03125 ml/l and above. Furthermore, at concentration of 0.5 ml/l of Bioact and ETOGROWTH, negative values of RGR and ECI were observed. When DBM was fed on the leaf discs treated with Bioact and ETOGROWTH, they consumed less food and gained less or even lost weight.

In addition, both Bioact and ETOGROWTH had fairly strong repellent effect on DBM at all concentrations tested. The values of repellent class were all above Class III.

Therefore, both Bioact and ETOGROWTH were more useful in the suppression of population development as a semiochemical to manage DBM rather than as a cidal compound in killing the pests in the IPM of modern farming.

Acknowledgements

We wish to express our thanks sincerely to Okada Ecotech Pte Ltd to provide us with the samples of tested insecticides.

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Table 1. Contact toxicity of Bioact-T EC6K and ETOGROWTH™ – EC (612) to larvae of *P. xylostella* *

Chemicals	LC_{50} (ml/l)	95% fiducial limits	LC_{95} (ml/l)	95% fiducial limits	Slope ± SE
Bioact-T **	1.689	0.579-5.976	356.564	43.46-252346	0.708±0.037
Etogrowth**	1.045	0.507-2.040	28.614	10.459-203.30	1.144±0.045

* The values were based on five concentrations, three replicates of 10 insects each.
** Bioact-T EC6K = Bioact-T EC6K ; Etogrowth = ETOGROWTH™ – EC (612).

Quality aspects of *Bacillus thuringiensis* products

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ABSTRACT
Bacillus thuringiensis (Bt) insecticidal products are manufactured around the world, with the main producers in the USA and China (CPL Business Consultants, 2010). A number of smaller producers can be found in India, Spain, Canada, S. Korea, Australia, Russia and a few other countries. As the largest global Bt manufacturer, Valent BioSciences Corporation periodically evaluates competitive Bt products marketed by others for quality and efficacy. This paper focuses on the results of recent tests conducted with a variety of such Bt products. Large differences between labeled and actual potencies have been found in Bt products for agricultural use, especially in products from Asia, Australia and Spain. Products with lower actual biopotencies had a remarkably lower efficacy in the field when compared to DiPel® at equivalent product rates. Growers not aware of such differences between labeled and actual potencies, who subsequently experience marginal crop protection may opt not to use a Bt product in the future. Maintaining Bt product quality is important for our industry, especially since Bt products represent a large portion of the biopesticide market and this market segment is slated to take an increasingly important role in pest control.

Keywords
Bacillus, *thuringiensis*, quality, biopotency, efficacy

INTRODUCTION
Commercial Bt products represent an important part of biorational product offerings for many companies worldwide. The term "biorational" when used to describe insecticidal products is reserved for products made from biological or natural origins For additional information on such products, please refer to www.growingproduce.com/biorational/. Bt products are manufactured using large-scale fermentation technology. Under optimal conditions of oxygen and nutrient supply, Bt cells produce insecticidal delta-endotoxin proteins in crystalline form and a spore. Bt strains used in commercial manufacturing have been selected for maximum production of delta-endotoxin proteins. Bacterial fermentation produces heat and as a result, fermentation vessels need adequate chilling equipment to stay within desired temperature parameters. Fermentation media preparation involves sterilization while maintaining nutrient value. Downstream processing for a dry Bt product requires drying/formulating of the fermentation slurry. All of these processing steps can have a major impact on Bt quality when not optimized. Quality assurance (QA) procedures ensure that every step of the manufacturing process is carried out as described in the manufacturing instructions. Quality control (QC) tests are performed during manufacturing and on the finished formulated product (Lisansky *et al.*, 1993). Documentation of the manufacturing process is part of the insecticide registration dossier in every country in which Bt products are sold.

In this paper, low quality commercial Bt products are defined as Bt formulated products with significantly reduced insecticidal activity, essentially showing far below label-stated potency as established in insect bioassays (biopotency). These products are not to be confused with low-biopotency labeled Bt products which are designed and registered as such by the manufacturer. What should be guarded against is allowing low quality Bt products to remain in the market place that promise sufficient pest control based on label potency statements but delivering insufficient amounts of Bt delta-endotoxins in the product. When growers use such low quality products and experience pest control well below expectations, it will cause a negative view of Bt products in general and create broader repercussions for the biopesticide industry.

All Bt products from Valent BioSciences Corporation (VBC) are fermented in a single manufacturing facility, and together with downstream processing and formulating all manufacturing steps are under tight control. VBC's Bt products are shipped to and distributed in 60 countries through Sumitomo Chemical Company (SCC) affiliated companies and a third party distribution network. As the largest Bt manufacturer in the world, VBC monitors the quality of Bt products in those markets where its products are distributed.

Quality aspects and Bt product registration
The US Environmental Protection Agency provides the framework for registering Bt products in the US and discusses with the manufacturer the data requirements that need to be met in order to conform with the health, safety and environmental guidelines for these types of products. Various components of the dossier include active ingredient (Bt) identity and method analysis, manufacturing and certification of ingredients, storage stability, and toxicity/pathogenicity results towards non-

target organisms. The documentation generated during manufacturing, such as batch records, manufacturing deviations and corrective actions, shipping receipts, inventory and disposal records are formally maintained and archived.

With regard to potency, however, the manufacturer sets Bt product potency and provides the data to EPA together with uses and labeled rates for the proposed final product label. No field efficacy data needs to be submitted to the EPA at the time of dossier filing, but of course field trial results are generated by the manufacturer in order to establish label rate recommendations. The manufacturer issues the product Certificate of Analysis (COA) to show the distributor that the product has the correct biopotency as listed on the EPA approved product label.

There are various levels of trust in this whole process. EPA does not retrieve Bt product samples from the market place for testing. The grower trusts that the manufacturer has tested the Bt product and it has met the product specifications reflected in the manufacturer's COA. The EPA trusts that the manufacturer has or has access to well established insect colonies, a well trained entomological staff and that biopotency results indeed represent the quality of the Bt product. The burden of proof of quality is in the market place, where the value of brand name status and grower satisfaction is well guarded.

VBC has a long history of manufacturing Bt products and performs QC using insect bioassays at the highest level of scientific ability. Three species of Lepidopteran insects (*Trichoplusia ni*, *Plutella xylostella* and *Spodoptera exigua*) are kept in large colonies. The first two species are standard insects for quality control on Bt subspecies *kurstaki* (Btk) and subspecies *aizawai* (Bta) products. In Figure 1, data are given for QC biopotency tests of 35 batches of the Bta product XenTari® over a time period of several years. The variability is the interplay between fermentation/formulation variability and insect bioassay variability, with the goal to have a final product biopotency of at least 15000 IU/mg or more in a *T. ni* bioassay.

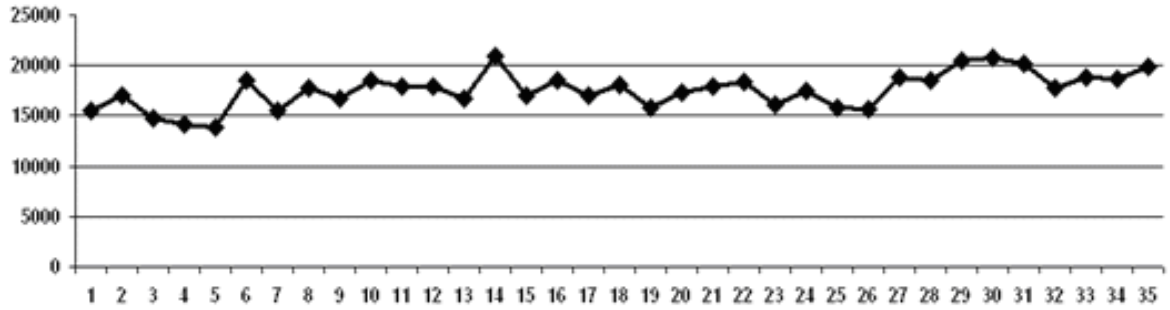

Figure 1. QC bioassay results from 35 large scale XenTari® production runs over several years, indicating consistency of delivering a potency of at least 15 000 IU/mg on cabbage looper, *Trichoplusia ni*. (Y-axis: potency as determined by insect bioassay; X-axis: individual production lots)

Effects of quality on product efficacy

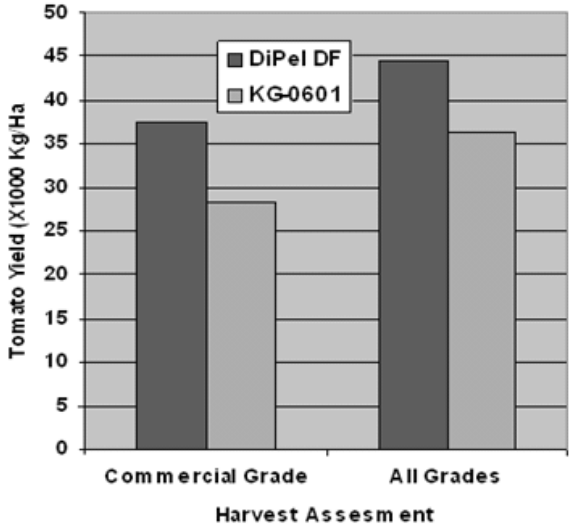

Figure 2. Tomato yield from one hectare plots treated with the Btk products, DiPel® DF versus KG-0601.

Using lower than labeled rates of any insecticide, Bt or chemical, will result in lower control of the target insect pest and poorer field efficacy. That is exactly what happens when low quality Bt products are applied at their labeled rate as there is just not enough Bt delta-endotoxin to cause sufficient insect mortality. Although the manufacturer used Bt fermentation solids in the formulation, it was not from a Bt fermentation manufactured under optimal conditions. Thus the finished product lacked a sufficient amount of insecticidal delta-endotoxin.

VBC monitors quality of Bt products in various regions of the world as part of our market evaluation efforts. As an example of this global monitoring program, product analysis and field trials were conducted in Spain. Trials were conducted on an organic tomato farm in one hectare plot with VBC's Btk product DiPel® DF (biopotency of 32000 IU/mg) at the rate of 1 kg/ha and compared with a local Btk product with the given code name of KG-0601. This product also had a labeled biopotency of 32000 IU/mg and was applied at the same rate of 1 kg/ha. After

five applications one week apart for control of *Helicoverpa armigera*, the tomatoes were harvested and total yield, as well as the culled yield of commercial grade tomatoes determined. Figure 2 shows that control of caterpillar damage was better with DiPel® DF as compared to KG-0601. Total tomato yield (all grades) as well as the commercial grade tomato yields were lower in the KG-0601 treatment.

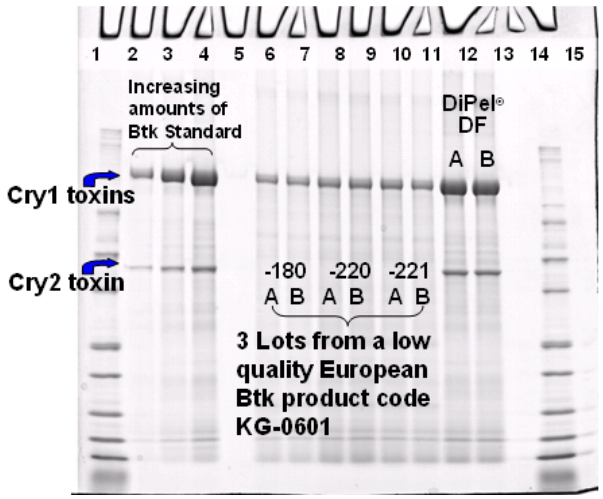

Figure 3. SDS-PAGE analysis of DiPel® DF and KG-0601 showing much reduced Bt delta endo-toxin content in KG-0601. A and B refer to two sample loadings of each lot.

Analysis of several lots of KG-0601 revealed that the level of Btk delta-endotoxin was much lower than that of our standard DiPel® DF (Figure 3). KG-0601 also showed a lower biopotency in insect bioassay. A total of six lots of this product have been tested and the average potency on *T. ni* is 10155 IU/mg product while the label potency states 32000 IU/mg product. Of course, companies can always refer to differences in insect bioassay methods, and to a certain extent that is correct. Insect bioassays done by different laboratories with different insect colonies and different Bt standards will show a certain level of difference in potency results. That is why the insect bioassay method with an additional chemical analysis brings more validity to evaluating Bt product quality and bioassays combined with a simple Bt delta-endotoxin analysis is a common approach. The analysis of Bt delta-endotoxins can be performed with a straight forward gel electrophoresis method called SDS-PAGE (Currier and Brussock, 1990). Although scientists are still debating the quantitative precision of the SDS-PAGE method, it is commonly accepted that a substantial decrease in the presence of Bt delta-endotoxin corresponds with lower biopotency and thus a lower field efficacy, as shown in our results on KG-0601. In short, the SDS-PAGE method offers a convenient first assay capability to evaluate Bt products.

In several Asian countries, the presence of low quality Bt products in the market place has been a constant problem despite the government monitoring efforts especially in Thailand and Taiwan (Kao and Tuan, 1995). As outlined above, Bt product quality can be documented by measuring the level of Bt delta-endotoxin. Table 1 lists the results of several Bt products manufactured in China and collected from several Asian countries. Invariably, the Bt products with low Bt delta-endotoxin content, as measured by SDS-PAGE analysis, have biopotencies below the labeled potency. The brand names are not given due to existing laws in various countries prohibiting negative comments about product brands.

Again the key issue is not about having Bt products with low potency in the market place, rather the problem is in having Bt products that have significantly lower biopotencies than what is on the label. Bt products with higher potencies command a higher price since they are more expensive to produce but are also more efficacious. A Bt product with a high potency label statement but with a significantly lower actual biopotency and Bt delta-endotoxin content will not deliver adequate field efficacy.

Table 1. Analysis of four Bt products manufactured in China. Note the reduced values of label stated potency and actual potency, as well as low amounts of Bt toxin protein in the Chinese products as opposed to DiPel® DF.

Product	Percent a.i. on label	Potency		Bt delta-endotoxin protein (mg/g)
		On label	Actual	
Chinese Bt #1	1.50	8000	2695	8.90
Chinese Bt #2	3.00	16000	3665	5.90
Chinese Bt #3	1.50	8000	5161	5.30
Chinese Bt #4	3.00	16000	9746	very low
DiPel® DF	6.40	32000	>32000	76.30

Low quality Bt products can be found in other parts of the world as well, such as Australia and Latin America. Even Bt products contaminated with chemical insecticides have been found in the market place by our VBC research team, referring to a specific case of a Btk product in Peru contaminated with chlorpyrifos. This is a grave situation since organic farmers rely mostly on Bt products for the control of caterpillar pests. Chemical insecticide residues on organic crops caused by contaminated Bt products can lead to revocation of organic certification and serious economic consequences for the farmer.

CONCLUSION

It is in the best interest of the biopesticide industry to identify and bring to light those instances where Bt products in the marketplace are found to possess significantly lower biopotencies than what is claimed on the label. The consistent manufacturing of high quality Bt products on a cost effective basis is a science that has taken years to perfect. A few large-scale Bt manufacturers, including VBC, have pioneered in this field of technology and have been providing high quality

Bt products with well recognized brand names to many farmers around the world. Since biocontrol products are such a small portion of the overall pest control market, not many individuals, governments, or companies pay attention to Bt product quality in the market place. Biocontrol products in general have a very good safety profile and instances of quality deviations are not a top priority for many in government or industry (with some exceptions). Finally, the expertise in analyzing Bt products is, again with a few exceptions, in the hands of Bt manufacturers. Therefore, in practice the Bt industry must self-monitor its products in the market place and call attention to those instances when products are not delivering upon their labeled biopotency claims. This paper has attempted to start that process.

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Antifeedant effect of *Acorus calamus* L. rhizome extracts against *Plutella xylostella* L.

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ABSTRACT
The antifeedant activity of *Acorus calamus* extracted by different organic solvents i.e. methanol, chloroform, benzene and hexane was assessed by choice and no-choice assays using thirds instars of *Plutella xylostella* (Plutellidae). It was observed that all *Acorus* extracts exhibited strong antifeedant activity based on a percent antifeedant index (AFI). Using a choice based assay, *Acorus* extracted in hexane showed the highest antifeedant index (96.65%) followed by methanol (90.91%), chloroform (89.68%); and benzene (73.79%) extracts. Using a no-choice based assay, *Acorus* methanol extract produced the highest antifeedant index value (77.58%) followed by chloroform (76.29%), benzene (69.65%), and hexane (68.53%) extracts.

Keywords
Acorus calamus L., rhizome, antifeedant, *Plutella xylostella* L.

INTRODUCTION
The roles of plant secondary metabolites are very important in their contribution to insect pest control because they can significantly affect insect behavior. Scientists believe that secondary metabolites produced by plants as defensive strategies are based on phytochemicals with hormonal, antihormonal, or toxic activities that originate from the continuous interaction between plant and insect, termed "co-evolution" (Gao et al. 2004). A variety of chemical components can act as insect antifeedants and also disrupt insect growth and reproduction (Koul et al. 2007). Species from 60 plant families have been identified as possessing insecticidal activity with 88 species reported to be active against *P. xylostella* (Morallo-Rejesus 1986; Dev and Koul 1997; Prakesh and Rao 1997).

The rhizomes of *A. calamus* (sweet flag) have attracted the attention of entomologists for their insecticidal properties against a wide variety of insects. The major chemical constituents of the essential oils of sweet flag are phenylpropanes, monoterpenes, and thermolabile sesquiterpenoids (Rost and Bos 1979, in Motley 1994.) All of those groups of chemical compounds are known for the antifeedant activity (Saxena and Koul 1978; El-Nahal, et al. 1989; Sharma et al. 2008), repellent activity (Saxena and Koul 1978; Paneru et al. 1997) and insect growth regular activity (Marthur and Saxena, 1975; Saxena et al. 1977; Nair and Thomas, 2001; Poplawski et al. 2001).

Recently, some researchers have reported the bioactivity of acorus extract or essential oil against agricultural pest. The most effective compound in *A. calamus* oil has been identified as β-asarone (cis-1-propenyl-2,4.5-trimethoxybenzene (Gildemeister and Hofmann, 1966; Schimdt and Streloke (1994). Cis-asarone or β-asarone posses antigonadal activity, which causes inhibition of egg production and other gonadal dysfunctions in *Dysdercus koenigii* F. (Saxena et al. 1977); dipterians (*Bactrocera cucurbitae* Coq.) (Nair and Thomas 2001), heteropterans, and coleopterans of both sexes (Saxena et al. 1977; Marthur and Saxena 1975; Poplawski et al. 2001). In addition, it has been reported that this compound inhibits growth and feeding in *Spodoptera litura* F. (Koul 1987) and *Peridroma saucia* Hub. (Koul et al. 1990). Cis-asarone has also been reported effective against S*itophilus oryzae* L., *Callobruchus chinensis* F. *and Lasioderma serricorne* L. (Park 2000 *in* Park 2003). However, Matsui et al. 1976 as reported by Sharma et al. 2008 showed that the trans-asarone or α-asarone does not induce any toxic effect but acts solely as a feeding deterrent.

Jiyavorranant et al. 2003 reported that ethanol extract of *A.calamus* rhizome at a concentration of 0.4% had the greatest activity against the larvae of *P. xylostella*. This concentration also caused 63.3% accumulated mortality with 23.33 mm^2 feeding sites per larva within 48 hours. Hossain et al. 2008 isolated a constituent of *A. calamus* rhizome from petroleum ether extract 2,4,5-trimethoxy benzaldehyde. This chemical substance possesses toxic activity against the adults of *Tribolium confusum* and *Sitophilus oryzae.* However, the toxicity of the methanol and acetone extracts of *Acorus* extracts on these stored product pests is low.

No published work on the effect of *Acorus* extracts from different organic solvents on *P. xylostella* (diamondback or cabbage moth) has been found. This present study was designed to examine the feeding response of diamond back larvae to extracts of *A. calamus* rhizome extracted with various solvents. The extracts were prepared with

the solvents hexane, ,benzene, chloroform and methanol. The results of the study are intended to be useful in promoting research aiming at the development of new agents for insect pest control based on bioactive chemical compounds from plants as an alternative to synthetic or other chemical insecticides.

MATERIALS AND METHODS

Plant material

Rhizomes of *A. calamus* was collected from the Jember district, East Java in Indonesia in December 2008 and was authenticated by Umiyah of the Biology department, laboratory of botany, University of Jember, Indonesia. The rhizome were cut thinly and dried at room temperature for one week. Dried rhizome (3 kg) was powdered mechanically using a commercial electrical stainless steel blender.

Insect rearing

P. xylostella rearing was done in the quarantine room and glasshouses of the School of Agriculture and Food Science, UQ Gatton. Rearing conditions were 14:10-h (light:dark) at 25±2°C and 54±4% relative humidity. Pupae were maintained in a quarantine room in wooden-frame gauze cages (ca. 40 cm x 40 cm x 40 cm) with 8-week-old broccoli seedlings and provided with 10% honey solution in cotton swabs for emerging adults. They were kept caged until adults emerged and mated. After the females have oviposited, seedlings with eggs were removed from the cages and kept until eggs hatched. Then, seedlings with newly-hatched larvae were transferred to the glasshouse. They were kept in the glasshouse until the larvae pupated. Pupae were harvested, then transferred to the laboratory, some for immediate emergence and mating, the rest kept at 4˚C until needed to maintain the population.

Preparation of plant extract

Extraction and fractionation of extracts from this plant were conducted in the Chemical Analytical Laboratory (CAL) UQ-Gatton. The powder of *A. calamus* (each of the 500 grams) was extracted using 2l of four organic solvents from least polar to the most polar (hexane, benzene, chloroform, and methanol) for 24 hours at room temperature. The mixtures were filtered using no. 1 Whatman filter papers and vacuum at 27 kPa on a Buchner funnel. Each of the combined filtrates was concentrated under reduced pressure at 40-50˚C in a flash evaporator at around speed 5 to yield about 50 ml of extract and the residue thus obtained was stored at 4˚C.

Antifeedant bioassay

During preliminary screening in laboratory trials, third instars of *P. xylostella* were collected from insect rearing cages held in the glasshouse. The stock solutions 10% (v/v) were prepared by mixing the crude oil extracts obtained with a dilutent, which became the control solutions and 0.5 ml emulsifier (Tergitol^{TM} XD (43%) and Tergitol TMN-10 (57%) 100 gm Net surfactant) except for chloroform extract, which required 0.1 ml of emulsifier. The control solution consisted of 10% of each solvent (hexane, benzene, methanol) and 0.5 ml emulsifier (Tergitol^{TM} XD (43%) and Tergitol TMN-10 (57%) 100 gm Net surfactant) in de-ionized water. For chloroform extract, the control solutions contained 10% of chloroform and 0.1 ml of emulsifier. Various concentrations of extracts were prepared by serial dilution of the stock solution with the control solution. The dipped leaf method (Park et al. 2003) was used to evaluate the antifeedant activity of the test samples.

A range of concentrations based on a preliminary range finding assay were applied. In this assay concentrations 2%, 4%. 6%, 8%, 10% and control (0%) were used. Each treated leaf disc (3 cm in diameter) was then placed on a tray and left in a fume cupboard for 2 h to evaporate the solvents. For each treatment, there were three replicate leaf discs; a total of 30 larvae were used in each treatment. The third instars of *P. xylostella* were exposed to the extracts using leaf disc choice and non-choice options. Prior to using larvae in the various bioassays, they were starved for 4 h. For the choice method, 10 larvae of the third instar were placed on treated and untreated/control leaf disks, which were arranged around a plastic container, while for the non-choice method, the treated leaf disks and controls were placed in separate plastic containers. After 24 h, larvae were removed and faeces brushed from the leaf disks, then the leftover leaf disks were placed on a piece of paper to digitize the form of the leaf disk with an Epson scanner set at 300 dpi. After that, image areas were measured by using a computer program, ImageJ 1.41o (open source program, http://rsb.info.nih.gov/ij). The antifeedant index for the choice method was calculated by means of the equations (C-T)/(C+T) * 100%, and for the non-choice method (C-T)/C *100%, where C and T (mm^2) denoted the consumed area of control and treated discs respectively (Simmonds et al. 1989).

Statistical analyses

Data of antifeedant indices from the choice and no-choice tests were analyzed by using an analysis of variance (ANOVA) and a general linear model (GLM) procedure with the SAS software package. A separate analysis was done for each compound. The differences between means were assessed using the Least Significant Difference (LSD) test. Result with $p \leq 0.05$ were considered to be statistical different (Zar 1993)

RESULTS AND DISCUSSION

Antifeedant index with choice methods

At first all the larvae to the treated leaf substrate showed repellence response and tried to move away from the feeding arena or on to the lid surface of the container.

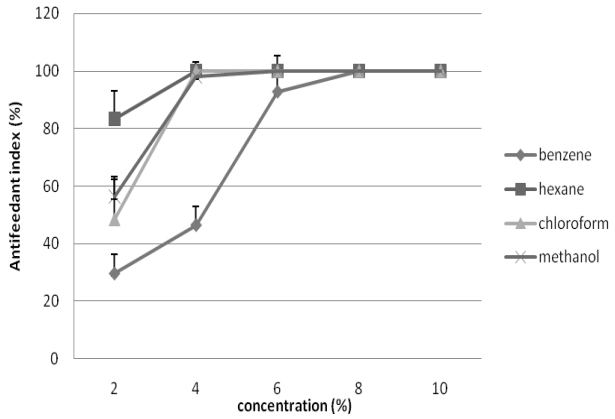

Figure 1. Concentration-antifeedant index relationship of *Acorus calamus* extract against third-instar *Plutella xyloxtella* with the choice method

Three hours after application, some of the larvae came close to the feeding arena, and were able to distinguish between the treated and untreated leaves. When untreated leaves discs were the first found, they settled and start to feed.

Table 1. Antifeedant effect of Acorus calamus extracted with four different organic solvents (benzene, hexane, chloroform, methanol) on third instars of Plutella xylostella in choice methods after 24 h, N:replication, AFI: Antifeedant index.

Solvent	Concentration (%)	N	AFI (Mean±sd) (%)	Pv; Pr>F	AFI interaction	Pv; Pr>F
Benzene	2	3	29.70±6.46c	69.73; 0.0001	73.79 ± 31.34c	50.99; 0.0001
	4	3	46.48±6.41b			
	6	3	92.81±12.45a			
	8	3	100 ± 0.00a			
	10	3	100 ± 0.00a			
Hexane	2	3	83.23±15.00b	3.75; 0.0409	96.65±8.97a	
	4	3	100 ± 0.00a			
	6	3	100 ± 0.00a			
	8	3	100 ± 0.00a			
	10	3	100 ± 0.00a			
Chloroform	2	3	48.39±5.79b	237.52; 0.0001	90.91±18.29b	
	4	3	100 ± 0.00a			
	6	3	100 ± 0.00a			
	8	3	100 ± 0.00a			
	10	3	100 ± 0.00a			
Methanol	2	3	56.4±9.72b	53.45; 0.0001	90.91±18.29b	
	4	3	98.16±3.18a			
	6	3	100 ± 0.00a			
	8	3	100 ± 0.00a			
	10	3	100 ± 0.00a			

The result of feeding choice assay showed that benzene, hexane, chloroform and methanol extracts of *A. calamus* acted as antifeedants. Koul 2007 stated that a plant chemical as antifeedant is considered to be effective when feeding inhibition of 80%-100% is achieved. The antifeedant activities were dose-dependent (Fig.1). The higher the rate applied, the higher the antifeedant index valued. Benzene extract of *A. calamus* at concentrations of 2%-6% reduced feeding by third instar *P. xylostella* larvae significantly (Table 1). However, at concentrations of 6%-10%, the antifeedant indices were not significant. At 2-6%, concentrations of the average antifeedant index in 24 h ranged from 29.70%-92.81%, while at 6%-10% the antifeedant indices were almost all 100%. At all rates, *P. xylostella* larvae preferred feeding on untreated leaves to treated ones. Hexane, chloroform and methanol extracts of *A. calamus* showed a similar pattern. At the rates 4%-10%, those *Acorus* extracts did not significantly reduce larval feeding. Those rates were significant only at the 2% level.

The interaction among the four different organic solvents of *Acorus* extract was significant ($F=50.99$, $df=4,14$, $p\leq0.0001$) as well as between the concentrations (*Acorus* in benzene: $F=69.73$, $df=4,14$, $p\leq0.0001$; *Acorus* in hexane: $F=3,75$, $df=4,14$, $p\leq0.0409$; *Acorus* in chloroform: $F=237.52$, $df= 4,14$, $p\leq0.0001$; *Acorus* in methanol: $F=53.45$, $df=4,14$, $p\leq 0.0001$). Benzene extract of *Acorus* had the lowest antifeedant index (73.79%) compared to 89.68%, 90.91%, and 96.65% for chloroform, methanol and hexane extracts respectively. The correlation values based on regression equation showed that benzene extract of *A. calamus* was higher than 0.81, 0.53, 0.5, and 0.5 for benzene, methanol, chloroform, and hexane extracts respectively) (Table 2).

Table 2. Acorus calamus extracts-Antifeedant index relationship against third-instar Plutella xyloxtella with choice method.

Acorus in solvent	Regression equations Y=	R^2
Benzene	19.41x + 15.56	0.81
Hexane	3.354x + 86.58	0.5
Chloroform	10.32x + 58.71	0.5
Methanol	8.904x + 64.2	0.53

Antifeedant index with no-choice method

Similarly to the choice method, at first, all the larvae exposed to the leaf substrate showed repellence action and tried to move away from the feeding arena or on to the lid surface of container. After 3 h of application, some of the larvae approached the feeding arena, and settled for about 30 min. It depended on the rate; the higher the rate, the longer they took to settle and start to feed.

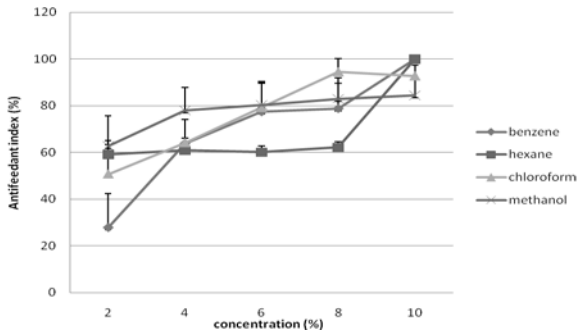

Figure 2. Concentration-antifeedant index relationship for *Acorus calamus* extracts against third-instar *Plutella xylostella* using the no-choice method.

The result from no-choice assays showed that benzene, hexane, choloform, and methanol extract of *A. calamus* actives as antifeedant. The antifeedant activity was dose-dependent (Fig. 2). The higher the rate applied, the higher the antifeedant activity index value. Benzene extract of *A. calamus* at concentrations of 2% and 10% significantly reduced feeding by *P. xylostella* third instars (Table 4) with other rates. However, at concentrations of 4%-8%, the antifeedant indices were not significant. At 2% and 10% the average antifeedant indices at 24 h were 27.98% and 100%, while at 4%, 6% and 8% the antifeedant indices were 63.92%, 77.49% and 78.87% respectively. At all rates, there was more feeding by *P. xylostella* larvae on control leaves than on treated ones. The antifeedant indices of hexane extract of *A. calamus* at 2%-8% were significant compared to 10% but were not significant among those rates. Chloroform extract of *A. calamus* at 2% and 4% showed significant effects on feeding reduction by the larvae at the higher rates (6%-10%) and for methanol extract of *A. calamus* the indices for 2%-6% differed significantly from 8% and 10%.

The interaction among the four different organic solvents of *Acorus* extract showed significance ($F=13.04$, $df=4,14$, $p\leq0.0001$) as well as between the concentrations (*Acorus* in benzene: $F=22.14$, $df=4,14$, $p\leq0.0001$; *Acorus* in hexane: $F=148.73$, $df=4,14$, $p\leq0.0001$; *Acorus* in chloroform: $F=11.44$, $df= 4,14$, $p\leq0.0009$) However, *Acorus* in methanol showed no significant difference among the rates: $F=2.25$, $df=4,14$, $p\geq0.1364$). Benzene and hexane extract of *Acorus* had the lower antifeedant indices (69.65% and 68.53%) than chloroform and methanol extract of *Acorus* extract (76.29%, 77.58% respectively). Non-polar solvent (benzene and hexane) indices differed significantly from polar solvent (chloroform and methanol) indices (Table 3). The correlation value based on regression analysis showed that chloroform extract of *A. calamus* was higher than the 0.927, 0.891, 0.771, 0.552 for benzene, methanol, chloroform, and hexane extract of *A. calamus* respectively (Table 4).

Table 3. Antifeedant effect of *Acorus calamus* extracted with four different organic solvents (benzene, hexane, chloroform, methanol) on third instars of *Plutella xylostella* in no choice methods after 24h, N:replication, AFI:Antifeedant index.

Solvent	Concentration (%)	N	AFI (Mean±sd) (%)	Pv; Pr>F	AFI interaction	Pv; Pr>F
Benzene	2	3	27.98±14.30c	22.14; 0.0001	69.65 ± 26.01b	13.04; 0.0001
	4	3	63.92±2.26b			
	6	3	77.49±12.4b			
	8	3	78.87±10.80b			
	10	3	100 ± 0.00a			
Hexane	2	3	59.21±4.01b	148.73; 0.0001	68.53±16.45b	
	4	3	60.94 ± 1.69b			
	6	3	60.17 ± 2.54b			
	8	3	62.26 ±2.42b			
	10	3	100 ± 0.00a			
Chloroform	2	3	50.79±14.24c	11.44; 0.0009	76.29±19.18a	
	4	3	64.18±10.02bc			
	6	3	79.21 ±10.31ab			
	8	3	94.50 ± 5.64a			
	10	3	92.78 ±4.5a			
Methanol	2	3	62.77±12.96b	2.25; 0.1364	77.58±11.71a	
	4	3	77.98±9.78ba			
	6	3	80.29 ±10.11ba			
	8	3	82.89 ± 8.94a			
	10	3	84.45 ± 7.61a			

Table 4. *Acorus calamus* extracts-antifeedant index relationship against third-instar *Plutella xylostella* with no-choice method.

Acorus in solvent	Regression equations Y=	R^2
Benzene	**15.89x + 21.95**	**0.891**
Hexane	**8.29x + 43.64**	**0.552**
Chloroform	**11.43x + 42**	**0.927**
Methanol	**4.836x + 63.18**	**0.771**

In general, the antifeedant indices of the test extracts in the choice method were greater than those in the no-choice assays. For the choice method, the average antifeedant index were ranged from 73.79%-100% after 24 h compared with no-choice methods, which ranged from 68.53%-77.58%. The no-choice situation is often more representative of an agricultural system. No other choice other than the treated leaves resulted in the larvae finally starting to eat the leaf tissue, apparently as a result of habituation response. This effect is a common response of many animals to repetition of the same stimulus, which can decrease their sensitivity (Koul 2005; Isman 1996).

CONCLUSION

A. calamus extracted in four different organic solvent (benzene, hexane, chloroform, and methanol) showed a potential effect in reducing the larvae feeding of *P. .xylostella* based on antifeedant index value. Both in choice and no-choice methods, all extracts can inhibit larvae feeding up to 68%. This antifeedant index increased significantly, up to 100% in the choice method. In general, based on the antifeedant index on the no-choice test, the crude *A. calamus* extracted in methanol used in this study was more potent than the other extracts.

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Antifeedant and toxicity activities of some botanical extracts and their chemical compounds against *Plutella xylostella* L. (Lepidoptera: Plutellidae)

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ABSTRACT
Antifeedant activities of *Duabanga grandiflora* crude methanol extract, *Diospyros cauliflora* crude chloroform extract and their chemical compounds, and the toxicity of both crude plant extracts were investigated on the third instar larva of Diamondback moth, *Plutella xylostella* under laboratory conditions. The crude methanol extract of *D. grandiflora* showed the highest antifeedant activity (100%), followed by crude chloroform extract of *D. cauliflora* (65.97%) at 8% (w/v) concentration with the AFI_{50} values of 1.14% and 4.23%, respectively after 24 h of treatment under choice conditions. The maximum deterred feeding of both crude extracts was found at the highest dose (8%), on choice and no choice bioassays. Three chemical compounds isolated from *D. grandiflora*, *viz*., *p*-hydroxybenzaldehyde, oleanolic acid and 4-*O*-α-L-rhamnopyranosyl-3'-methoxyellagic acid at 0.5% concentration exhibited high feeding deterrence recording 56.15%, 54.81% and 48.83%, respectively at 24 h after exposure, whereas maximum feeding deterrence of nicotinamide, an isolated compound of *D. cauliflora* was only 22.65%. The crude methanol extract of *D. grandiflora* and crude chloroform extract of *D. cauliflora* had no toxicity on third instar *P. xylostella* larvae when compared to the control at 24, 48 and 72 h after exposure.

Keywords
Duabanga grandiflora; *Diospyros cauliflora*; *Plutella xylostella*; antifeedant; toxicity

INTRODUCTION
The diamondback moth (DBM), *Plutella xylostella* L. (Lepidoptera: Plutellidae) is a specialist pest of cruciferous crops in the family Brassicaceae throughout the world (Akhtar and Isman 2003). The larvae feed from seeding stage to harvest, diminishing quality and quantity of yield (AVRDC 1992; Asma 1994). This insect causes more than 90% crop loss in cabbage (Oke 2008). Population of DBM is very hard to control due to its short life cycle, high reproductive rate and active flying to find the new food sources (Schoonhoven 1993).

Synthetic pesticides are the primary tactic for controlling this pest due to its convenient application and quick response. For example, organophosphates, carbamates, pyrethroids, insect growth regulators, abamectins, pyrazoles, oxadiazines and neonicotinoids were used to control the populations of DBM in many countries (Mohan and Gujar 2003; Charleston et al. 2005; Abdel-Razek et al. 2006; Qian et al. 2008).

However, application of pesticides in agriculture to control various insects and mites not only have a positive effect for controlling pests, crop diseases and weeds, but also resulted in a variety of problems such as pesticide residues in food chain, the effects of pesticides on the natural enemies and pests becoming resistant to many classes of pesticides (WCED 1988; Metcalf 1989; Sastroutomo 1992; UNEP 1992). Consequently, the development of control techniques that provides efficient pest control without serious effects on product, environment and public health are required.

A botanical pesticide can be employed as an alternative source to control pests with biodegradable concern, reductive contamination in environment and human health hazards (Grainge and Ahmed 1988; Devlin and Zettel 1999). Pyrethrins, rotenone and nicotine were among the first compounds from plants used to control agricultural insect pests (Grainge and Ahmed 1988). Botanical pesticides are also special because they can be produced easily by farmers for sustainable agriculture and small industries (Roy et al. 2005). Many plant species are being investigated for their natural products to be used for *P. xylostella* control. For instance, *Azadirachta indica* A. Juss. (Meliaceae), *Melia azedarach* L. (Meliaceae) and *Acorus calamus* L. (Araceae) treatments were found to inhibit feeding of *P. xylostella* 24 h after treatment (Patil and Goud 2003).

Duabanga grandiflora (Roxb. ex DC.) Walp., which belongs to subfamily Duabangoideae, family Lythraceae is a member of a tropical African and Southeast Asian tree (Graham et al. 1998; 2005). The hill tribe people in the northern Thailand use the poultices from its leaves to treat stomach pains (Anderson 1986). The broth-based (turbidometric, TB) assay showed the inhibitory effects

of *D. grandiflora* on the growth kinetic of the bacteria, *Escherichia coli* and *Staphylococcus aureus* (Othman et al. 2011). The timber methanol extract of *D. grandiflora* was also examined for its leishmanicidal activities and revealed less inhibition (Takahashi et al. 2004).

Diospyros cauliflora Blume belongs to genus *Diospyros*, which is a large genus of mainly tropical trees within family Ebenaceae. Many of which possess considerable economic importance such as edible fruits (persimmons, *Diospyros kaki* Thunb and *Diospyros virginiana* L.) and ebony (*Diospyros ebenum* J. Koenig). The bark extract (dichloromethane:methanol, 1:1) of *D. cauliflora* was tested on malarial parasite, *Plasmodium falciparum* (Welch, 1897) (Gombak A: chloroquine-resistant strain and D 10: chloroquine-sensitive strain) and revealed parasite inhibition at > 50 ug/ml (Khozirah et al. 2011).

As the information dealing with insecticidal activity of *D. grandiflora* and *D. cauliflora* are still lacking, it is interesting to study their biological effects on *P. xylostella* under laboratory conditions. The information from such studies not only increase the knowledge in bioinsecticidal properties of these plant species, but also can be used as an alternative way to control such insect which is safe for human life and the environment.

MATERIALS AND METHODS

Plant materials

Stem branches of *D. grandiflora* were collected in Kanchanaburi province located in the western Thailand in January 2007. The plant material was identified by Mr. Pranai Penchit and the herbariums specimen (CHKU 00010) was deposited at the Bangkok Herbarium Botanical Research Unit, Plant Variety Protection Division, Department of Agriculture, Bangkok, Thailand. Roots of *D. cauliflora* were collected in Trang province, southern Thailand in March 2007 and identified by Mr. Chamlong Phengklai. A voucher specimen (BKF 143220) has been deposited at the Royal Forest Department, Phaholyothin Road, Bangkok, Thailand.

Preparation of plant extracts

The stem branches of *D. grandiflora* (10 kg) were machine-cut into small pieces and dried at 40ºC in hot air oven before grinding into power. Dried powder (5 kg) was extracted with methanol 3×20 L at room temperature. The methanolic solution was filtered through a Whatman No.1 filter paper and concentrated by a rotary evaporator under reduced pressure to give crude methanol extract (190 g). Dried root powder of *D. cauliflora* (4 kg) was extracted with hexane (3 x10 L) at room temperature. The hexane solutions were combined and evaporated under reduced pressure to give a crude hexane extract (23 g). The residue was then extracted with mathanol (3x10 L) at room temperature for 7 d. The methanolic solutions were combined and evaporated under reduced pressure to give dark brown syrupy mass of crude methanol extract (130 g). The crude methanol extract was then dissolved, with sonication, in chloroform (3x1 L), and filtered. The solutions were combined and evaporated under reduced pressure to give crude chloroform extract (26 g).

Pure compounds

Six pure compounds isolated from crude chloroform extract of the stem branches of *D. grandiflora* (*p*-hydroxybenzaldehyde, 6H-dibenzo[*b,d*]pyran-3,9-dihydroxy-6-one, arjunolic acid, 3-glycosyl-β-sitosterol, acacetin 7-*O*-glucoside and 4-*O*-α-L-rhamnopyranosyl-3'-methoxyellagic acid) were used in this study. Moreover, seven pure compounds (oleanolic acid, acacetin, apigenin, betulinic acid, vanillic acid, nicotinamide and lupeol) were purchased from Sigma-Aldrich chemical company (St Louis, MO), and used in this study. The first three compounds were found in *D. grandiflora* extract, whereas betulinic acid and vanillic acid appeared in both crude extracts. Nicotinamide and lupeol are found in roots of *D. cauliflora* crude chloroform extract.

Test insects

The stock colonies of *P. xylostella* used in this study were collected from cruciferous plants in many provinces of Thailand. The larvae were reared on organic Chinese mustard (*Brassica chinensis* Jusl var *parachinensis* (Bailey) Tsen&Lee) leaves in plastic box under laboratory condition at 25±1ºC, Department of Entomology, Faculty of Agriculture, Kasetsart University, Thailand. The colonies had been maintained continuously for several generations before they were used for bioassay. All bioassays were conducted under the same environmental conditions as the culture.

Antifeedant bioassay

Leaf disc choice test

Leaf disc choice test was carried out to evaluate the antifeedant activity of crude methanol extract of *D. grandiflora*, crude chloroform extract of *D. cauliflora* and their pure compounds against third instar *P. xylostella* larvae. Adequate amounts of crude *D. grandiflora* methanol extract and crude *D. cauliflora* chloroform extract were dissolved in 70% ethanol and 95% ethanol, respectively to obtain 0.5, 1, 2, 4 and 8% (w/v) solutions. Besides, the pure compounds were dissolved in 95% ethanol, methanol or dichloromethane to prepare 0.5% (w/v) concentration. Aliquot of 15 µl of each tested material was dropped on the leaf disc (1 cm diameter) and smeared to cover all leaf arenas before allowing to dry completely at room temperature. A control disc was prepared in the same manner by using solvent.

A choice test was performed in a 9 cm diameter plastic Petri dish lined with moistened cotton pads and damped straw paper. The arena was divided into two equal parts. One Petri dish contained a treated and control leaf discs which were placed alternately. One third instar *P.*

xylostella larva (after starvation for 4 h) was introduced into the center of each Petri dish before wrapping with plastic wrap. Each treatment was replicated 15 times. The larvae were allowed to feed on the leaf discs for 24 h. A modified leaf area meter were used to calculate the amount of leaf area consumed by the larvae and the antifeedant index was calculated using the formula AFI = (C-T)/(C+T)×100, where: C = control leaf areas consumed by the insect and T = treatment leaf areas consumed by the insect (Isman et al. 1990).

No choice test

The no choice leaf disc test was carried out in exactly the same manner as leaf disc choice, except that the larvae were provided with only one leaf disc. The leaf discs were treated on their upper surfaces with either tested material or solvent as mentioned above. After the treated materials or solvent were evaporated completely under room temperature, a leaf disc was placed in a plastic rearing cup lined with moistened cotton pads and damped straw paper. After that, the plastic lid with net cover was placed on the plastic rearing cup. One starved third instar *P. xylostella* larva was allowed to feed on each leaf disc for 24 h. There are 15 replications for each treatment. The leaf consumption rate was measured using a modified leaf area meter.

Toxicity bioassay

A direct spray method was used to determine toxicity of the crude methanol extract of *D. grandiflora* and crude chloroform extract of *D. cauliflora* on third instar *P. xylostella* larvae. Ten larvae were randomly picked and released to the center of a glass Petri dish (9 cm diameter) before they were sprayed with 500 µl of 0.5, 1, 2, 4 or 8% of *D. grandiflora* or *D. cauliflora* crude extracts using a hand sprayer. Control received the same volume of 70% or 95% ethanol which were used to dissolve the crude methanol extract of *D. grandiflora* and crude chloroform extract of *D. cauliflora*, respectively. After the test materials or solvent completely evaporated at room temperature, the Chinese mustard leaf (3×3 cm) was provided as food source in each Petri dish before the dish was wrapped with plastic wrap. There were three replications for each treatment. Mortality of the larvae was recorded 24, 48 and 72 h after treatment and the Chinese mustard leaf was changed daily.

Statistical analysis

Antifeedant and toxicity activities were analyzed by one way analysis of variance. Significant difference between treatments was compared by Least Significant Difference (LSD) ($\alpha = 0.05$) test using SAS program (SAS Institute Inc., Cary, NC). AFI_{50} values were calculated based on AFI values at each dose using regression model of SPSS software. Percent leaf consumption between control and treatment was also compared using paired *t*-test.

RESULTS AND DISCUSSION

Antifeedant bioassay

The antifeedant activities of *D. grandiflora* and *D. cauliflora* crude extracts 24 h after treatment using leaf disc choice and no choice test against third instar *P. xylostella* larvae were given in Table 1. In leaf disc choice test, both crude extracts showed antifeedant activity at all concentrations except 0.5% of *D. cauliflora* crude chloroform extract -(9.94%). The antifeedant activity tended to increase with dosage. Among five concentrations of the crude methanol extract of *D. grandiflora*, the highest concentration (8%) showed highest antifeedant index of 100%, whereas the lowest concentration (0.5%) gave only 42.25% AFI which was significantly different from 4 and 8%. On the other hand, the crude chloroform extract of *D. cauliflora* at lowest concentration (0.5%) showed no antifeedant index (-9.94) but the highest concentration (8%) exhibited 65.97% antifeedant index.

Both crude plant extracts showed potential antifeedant activity to third instar *P. xylostella* larvae, but the larva is more susceptible to the crude methanol extract of *D. grandiflora* than crude chloroform extract of *D. cauliflora*. This fact can be verified by the effective concentration for 50% antifeedant activity of crude extract compared to the control (AFI_{50}) where the AFI_{50} values for crude methanol extract of *D. grandiflora* and crude chloroform extract of *D. cauliflora* were 1.14% and 4.23%, respectively. This result can imply that crude methanol extract of *D. grandiflora* possessed more antifeedant activity than crude chloroform extract of *D. cauliflora* under choice test. In general, *P. xylostella* larvae consumed control leaf disc more than treated leaf disc at all concentrations for both crude plant extracts. However, the consumption of control leaf disc by larvae was significantly different from the consumption of treated leaf disc at 0.5% ($P = 0.0083$), 2% ($P = 0.0255$), 4% ($P = 0.0020$) and 8% ($P = 0.0006$) for crude methanol extract of *D. grandiflora* and 4% ($P = 0.0170$) and 8% ($P = 0.0022$) for crude chloroform extract of *D. cauliflora*.

In no choice test, the maximum antifeedant activity on third instar *P. xylostella* larvae was showed for 8% crude methanol extract of *D. grandiflora* since the larvae consumed only 3.02% of the treated leaf disc, which is significantly less than control disc. Likewise, *P. xylostella* larvae fed with 8% crude chloroform extract of *D. cauliflora* consumed only 14.82% of the treated leaf disc. However, percentage of leaf consumption by larvae fed with 2, 4 and 8% *D. cauliflora* crude extract was significantly different from those fed with control disc. It seemed that crude chloroform extract of *D. cauliflora* was more effective than crude methanol extract of *D. grandiflora* in possessing the antifeedant activity against *P. xylostella* larvae under no choice test. The crude chloroform extract of *D. cauliflora* started to inhibit feeding of *P. xylostella* larvae at 2% concentration whereas crude methanol extract of *D. grandiflora* can inhibit feeding at the highest concentration (8%) only. However, the relationship

between antifeedant activity and concentration of crude extracts were not clear in this study.

The results demonstrated that both crude plant extracts showed best antifeedant activity on third instar *P. xylostella* larvae at 8% concentration, under choice and no choice conditions. Moreover, the antifeedant activity of both crude plant extracts was directly proportional to the concentrations of the extracts under choice test. The same trend was seen in crude methanolic extract of *Adhatoda vasica* Nees leaves (Acanthaceae) against *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) (Sadek 2003). In his choice test, at concentrations of 0.01, 0.1 and 0.2% crude methanol extract of *A. vasica* were tested with sixth instar larvae of *S. littoralis* and 16.4, 63.4 and 90.4% AFI were recorded at 4 h. In addition, the consumption rate of control leaf discs was significantly higher than the treated leaf discs in all tested concentrations (Sadek 2003). Similarly, there was a direct relationship between concentration of *n*-hexane, chloroform and ethyl acetate extract of *Acalypha fruticosa* Forssk leaves (Euphorbiaceae) and feeding deterrence against third instar *P. xylostella* larvae at 24 h after treatment using no choice test (Lingathurai et al. 2011). The *n*-hexane showed antifeedant activity of 12.5, 23.2, 29.6 and 41.4% at 0.625, 1.25, 2.5 and 5% concentrations, respectively. The crude chloroform extract revealed antifeedant activity of 25.8, 42.7, 78.3 and 92.8% at 0.625, 1.25, 2.5 and 5% concentrations whereas the crude ethyl acetate extract showed antifeedant activity of 19.2, 26.5, 50.6 and 64.0% at 0.625, 1.25, 2.5 and 5% concentrations, respectively. However, the crude chloroform extract of *A. fruticosa* leaves possessed more effective antifeedant activity than hexane and ethyl acetate treatments (Lingathurai et al. 2011).

The antifeedant activity of the crude methanol extract of *D. grandiflora* and crude chloroform of *D. cauliflora* against third instar *P. xylostella* larvae is probably due to the additive effect of active compounds in the extracts. The result of isolated compounds from both crude extracts on *P. xylostella* larvae using leaf disc choice test was shown in Table 2. At 24 h after treatment, the maximum antifeedant activity was found at 0.5% of *p*-hydroxybenzaldehyde (56.15%) followed by oleanolic acid (54.81%) and 4-*O*-α-L-rhamnopyranosyl-3'-methoxyellagic acid (48.83%), 3-glycosyl-β-sitosterol (23.37%) and nicotinamide (22.65%). On the contrary, betulinic acid, vanillic acid, acacetin, acacetin 7-*O*-glucoside showed no antifeedant activity whereas other isolated compounds showed antifeedant activity ranging between 5-12%. Only *p*-hydroxybenzaldehyde (P = 0.0047), oleanolic acid (P = 0.0208), 4-*O*-α-L-rhamnopyranosyl-3'-methoxyellagic acid (P = 0.0453) and acacetin (P = 0.0043) showed significant differences between the consumption rates of control and treated leaf discs by *P. xylostella* larvae.

Feeding deterrent activity of some isolated compounds from both crude extracts against *P. xylostella* and other insects has already been reported (Argandona and Faini 1993; Jagadeesh et al. 1998; Bhakuni et al. 2002; Huang et al. 2008). Oleanolic acid has been reported as an antifeedant to *Heliothis zea* L. (Lepidoptera: Noctuidae) larvae (Argandona and Faini, 1993). Betulinic acid which was extracted from crude methanol of *Zizyphus xylopyrus* Gotti showed active feeding deterrence only at a higher dose (150 μg/cm^2) against fourth instar *Spodoptera litura* F. (Lepidoptera: Noctuidae) larvae under no choice assay (Jagadeesh et al. 1998). The derivatives of the betulinic acid structure by the addition of a methylcinnamic, methoxycinnamic, or tri-*O*-methylgallic acid moiety in the C-3 position of the betulinic acid resulted in increased antifeedant property of the compounds against *S. litura* larva (Jagadeesh et al. 1998). Huang et al. (2008) reported that acacetin, isolated compounds from *Ajuga nipponensis* Makino (Labiatae) showed significant antifeedant activities with AFI_{50} values of 6.59 mg/ml after 24 h and 2.58 mg/ml after 48 h of treatment against third instar larvae of *P. xylostella*. On the contrary, apigenin the isolated compound from *A. nipponensis* showed a strong attraction to *P. xylostella* larvae with AFI value of -46.8 and -19.35% at 2000 ug/ml after 24 and 48 h after treatment. Bhakuni et al. (2002) revealed that arjunolic acid isolated from the stem of *Cornus capitata* Wall. ex Roxb. (Cornaceae) demonstrated an effective concentration (EC_{50}) to inhibit 50% feeding and growth of fourth instar *Spilarctia oblique* Walker (Lepidoptera: Arctiidae) larvae at 617.81 and 666.97 ppm at 72 h after treatment. In our experiments, we also found that oleanolic acid showed antifeedant activity against third instar *P. xylostella* larvae which is in agreement with these findings.

Toxicity bioassay

The toxicity of crude methanol extract of *D. grandiflora* and crude chloroform extract of *D. cauliflora* against third instar *P. xylostella* larvae is illustrated in Table 3. No toxic effects were recorded for both crude extracts on *P. xylostella* larvae at any observation time when compared to the control. Furthermore, the tested concentrations did not vary in percent mortality of the larvae. The result indicated that both crude plant extracts act mainly as feeding deterrent on third instar *P. xylostella* larvae, but did not show toxicity when the materials were treated directly on the larvae. Similar results were earlier reported in some plant extracts; for example the crude methanol extract of *Trichilia americana* (Sesse and Mocino) Pennington showed antifeedant activity (50%) at 0.18 ug/cm^2 against *S. litura* but it was not toxic (Wheeler and Isman 2001). The neem triterpenoids possessed antifeedant activity to *S. littoralis* without any toxicity (Aerts and Mordue 1997). Usher and Feeny (1983) also revealed that cucurbitacins, cardenolides and alkaloids deterred *Pieris rapae* L. (Lepidoptera: Pieridae) larvae but did not show toxicity for this insect. Moreover, hexane oil and crude hexane extracts of *Melaleuca leucadendron* L. showed deterred feeding of *S. litura* larvae in leaf disc choice test while *S. litura* larvae, tropically treated with 10%

crude extracts of this plant showed no toxicity activity (Kongkathip et al. 2004).

CONCLUSION

The current study demonstrated the antifeedant activity of crude methanol extract of *D. grandiflora* and crude chloroform extract of *D. cauliflora* against third instar *P. xylostella* larvae. The maximum feeding deterrence for both plant extracts were found at the highest dose. Three isolated compounds from *D. grandiflora* (*p*-hydroxybenzaldehyde, oleanolic acid and 4-*O*-α-L-rhamnopyranosyl-3'-methoxyellagic acid) act as antifeedant compounds on *P. xylostella* larvae. Likewise, nicotinamide isolated form *D. cauliflora* demonstrated antifeedant activity. The crude methanol extract of *D. grandiflora* and crude chloroform extract of *D. cauliflora* had effective feeding deterrence to the third instar *P. xylostella* larvae but did not present any toxic effects when sprayed directly on the *P. xylostella* larvae. Many isolated compounds from both crude extracts showed no antifeedant activity or slightly attractive to third instar *P. xylostella* larvae. These compounds can be mixed with pesticides for controlling the insect since the compounds can attract *P. xylostella* larvae to consume the leaf with pesticides which will later cause death to the insect. Hence, these botanical extracts and some isolated compounds needed to be investigated in more detail in order to use as alternatives to control *P. xylostella* larval population.

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Table 1. Antifeedant index (AFI) and leaf consumption (%) of third instar *Plutella xylostella* larvae fed with crude methanol extract of *Duabanga grandiflora* or crude chloroform extract of *Diospyros cauliflora* for 24 h in choice and no choice bioassays

Plant extracts	Treatments	Leaf disc choice bioassay		No choice bioassay [1/]
		Antifeedant index [1/]	Leaf consumption (%) [2/]	
D. grandiflora (Methanol extract)	0.5%	42.25 ± 22.04 b	85.77/14.23 *	8.64 ± 3.04 ab
	1%	48.81 ± 22.68 ab	77.81/22.19 ns	10.90 ± 4.18 ab
	2%	55.91 ± 20.87 ab	80.78/19.22 *	19.13 ± 8.05 a
	4%	95.55 ± 3.24 a	96.87/3.13 *	11.89 ± 6.88 ab
	8%	100.00 ± 3.70 a	100.00/0.00*	3.02 ± 1.29 b
	Control	NA	NA	21.76 ± 5.93 a
	F, df, P	2.22, 4, 0.0784		1.62, 5, 0.1633
	AFI_{50} (Confidence limits)	1.143% (-0.054 - 1.871)		
D. cauliflora (Chloroform extract)	0.5%	-9.94 ± 24.17 b	57.24/42.76 ns	59.91 ± 10.18 a
	1%	37.01 ± 22.89 ab	60.38/39.62 ns	37.55 ± 10.32 ab
	2%	47.55 ± 21.37 ab	70.16/29.84 ns	22.79 ± 8.89 b
	4%	49.43 ± 20.29 ab	71.71/28.29 *	30.21 ± 9.17 b
	8%	65.97 ± 19.43 a	89.74/10.26 *	14.82 ± 7.56 b
	Control	NA	NA	59.54 ± 9.03 a
	F, df, P	1.73, 4, 0.1550		4.15, 5, 0.0020
	AFI_{50} (Confidence limits)	4.233% (3.123 - 5.546)		

[1/] Means ± SE of untransformed data in column followed by the same letters are not significantly different as determined by Least Significant Difference test (LSD) (α = 0.05).
[2/] Leaf consumption (%) in control/treated discs, * indicated significant difference between control and treated leaf discs consumed by larvae at $p < 0.05$ and ns indicated non-significant difference at $p > 0.05$ by pair *t*-test.

Table 2. Antifeedant index (AFI) and leaf consumption (%) of third instar larvae of *Plutella xylostella* fed with isolated compounds of *Duabanga grandiflora* and *Diospyros cauliflora* for 24 h in choice bioassay

Individual compounds (0.5%)	Choice bioassay	
	Antifeedant index [1/]	Leaf consumption (%) [2/]
p-hydroxybenzaldehyde [3/]	56.15 ± 14.91 a	75.58/24.42 *
betulinic acid [3/, 4/]	-7.11 ± 23.94 bcd	44.44/55.56 ns
oleanolic acid [3/]	54.81 ± 21.70 ab	81.30/18.70 *
vanillic acid [3/, 4/]	-4.46 ± 24.47 abcd	51.47/48.53 ns
acacetin [3/]	-48.08 ± 16.81 d	23.68/76.32 *
apigenin [3/]	12.03 ± 24.07 abcd	54.38/45.62 ns
6H-dibenzo[*b,d*]pyran-3,9-dihydroxy-6-one [3/]	5.33 ± 24.92 abcd	67.34/32.66 ns
arjunolic acid [3/]	8.37 ± 22.87 abcd	63.20/36.80 ns
3-glycosy-β-sitosterol [3/]	23.37 ± 22.01 abc	50.10/49.90 ns
acacetin 7-*O*-glucoside [3/]	-2.93 ± 26.38 abcd	44.44/55.56 ns
4-*O*-α-L-rhamnopyranosyl-3'-methoxyellagic acid [3/]	48.83 ± 24.05 ab	78.77/21.23 *
nicotinamide [4/]	22.65 ± 22.02 abc	67.89/32.11 ns
lupeol [4/]	-16.60 ± 18.83 cd	32.95/67.05 ns
F, df, P	1.80, 12, 0.0529	

[1/] Means ± SE of untransformed data in column followed by the same letters are not significantly different as determined by Least Significant Difference test (LSD) (α = 0.05).
[2/] Leaf consumption (%) in control/treated discs, * indicated significant difference between control and treated leaf discs consumed by larvae at $p < 0.05$ and ns indicated non-significant difference at $p > 0.05$ by pair *t*-test.
[3/] Compounds isolated from *Duabanga grandiflora*.
[4/] Compounds isolated from *Diospyros cauliflora*.

Table 3. Percent mortality of third instar larvae of *Plutella xylostella* after spraying with crude methanol extract of *Duabanga grandiflora* and crude chloroform extract of *Diospyros cauliflora* 24 h, 48 h and 72 h after exposure

Plant extracts	Treatments	Mortality (%) [1/]		
		24 h	48 h	72 h
D. grandiflora (methanol extract)	0.5%	3.33 ± 3.33 ab	10.00 ± 0.00 ab	23.33 ± 6.67 a
	1%	13.33 ± 6.67 a	26.67 ± 8.82 a	26.67 ± 8.82 a
	2%	6.67 ± 3.33 ab	16.67 ± 6.67 ab	30.00 ± 5.77 a
	4%	0.00 ± 0.00 b	6.67 ± 6.67 b	26.67 ± 17.64 a
	8%	3.33 ± 3.33 ab	10.00 ± 0.00 ab	16.67 ± 3.33 a
	Control	3.33 ± 3.33 ab	13.33 ± 8.82 ab	23.33 ± 14.53 a
D. cauliflora (chloroform extract)	0.5%	10.00 ± 10.00 a	10.00 ± 10.00 a	16.67 ± 12.02 a
	1%	3.33 ± 3.33 a	13.33 ± 3.33 a	20.00 ± 5.77 a
	2%	3.33 ± 3.33 a	10.00 ± 5.77 a	13.33 ± 3.33 a
	4%	3.33 ± 3.33 a	13.33 ± 8.82 a	23.33 ± 18.56 a
	8%	0.00 ± 0.00 a	0.00 ± 0.00 a	20.00 ± 10.00 a
	Control	0.00 ± 0.00 a	10.00 ± 0.00 a	20.00 ± 5.77 a

[1/] Mean ± SE of untransformed data in column followed by the same letters are not significantly different as determined by Least Significant Difference test (LSD) (α = 0.05)

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Field evaluation of insect exclusion netting for the management of pests on cabbage *(Brassica oleracea* var. *capitata)* in the Solomon Islands

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ABSTRACT

This research evaluated the efficacy and financial feasibility of using exclusion row cover netting to exclude insect pests from cabbage crops as part of a management strategy. Two net materials, Evolution® Row Cover and MikroKlima® GrowCover were compared with the local practice (where insects are picked by hand or no control), at three locations in the Solomon Islands. The use of Evolution® Row Cover and MikroKlima® GrowCover resulted in 72% and 38% less pest damage compared to the local practice. There was little difference in size and weight of the heads harvested between treatments but there was an average increase of 40% in market price due to better quality heads grown under the MikroKlima® GrowCover. Although the Evolution® Row Cover provided the better protection, it was less durable and more easily damaged than the MikroKlima® GrowCover and needed regular repairs. Based on a predicted use of the MikroKlima® GrowCover for six crop cycles and the nature of the market at the time, the net present value for the Mikroklima® net treatment in Busarata was SBD 1,387.68 From the results of this study, there is a justification, both from production and financial perspectives, for using insect exclusion netting on high value crops in Solomon Islands, particularly if a cheaper source of durable netting can be found.

Keywords

Plutella xylostella, floating row covers, economic evaluation, pest control

INTRODUCTION

Vegetable growing in the Solomon Islands is predominantly small-scale production, either in home gardens or in small plots, and local, readily available inputs are used. Ball (=head) cabbage is a high value, cash crop for many farmers. The main consumers of ball cabbage are expatriates and the food service sector, where they are mainly used in salads. The crop is in high demand but there are many production constraints and crop quality (and hence market prices) are poor; production is restricted to a small number of farmers in the highlands of Malaita province, where production is still profitable.

Income generated from the sale of vegetables is important to rural households. In a baseline survey conducted in two provinces in 2008, 53% of the total income was through vegetable sales (Siliota et.al. 2008). Ball cabbage is a valuable income generator for farmers, as it is easily transportable, and has a relatively long shelf-life, compared to other vegetable crops. In addition, direct markets, such as hotels, restaurants and resorts, are showing increased interest in buying locally produced vegetable crops. However, these markets demand consistent and high quality supplies, but neither of these requirements is easily met. Consequently, significant quantities of cabbage are imported from Australia and New Zealand.

In Solomon Islands, the vast majority of vegetable production is organic, with the few farmers that use pesticides mainly targeting Lepidopteran pests. It is, therefore, important to find alternative solutions to managing pest populations, without developing a reliance on the use of synthetic insecticides.

Diamondback moth (*Plutella xylostella*) and other *Brassica* pests, including Cabbage cluster caterpillar (*Crocidolomia pavonana*) and common armyworm (*Spodoptera litura*) are major pests of ball cabbage *(Brassica oleracea var. capitata)* in the Solomon Islands. The population levels of species such as *P. xylostella* are significant enough to limit the production of ball cabbage to a few locations, mainly in the highlands. However, with the increase in production of watercress *(Rorippa nasturtium-aquatica)* in these areas, population levels of *Brassica* pests are becoming an increasing restricting factor for producers. Watercress is also an important food crop to the Solomon Islands, but is only grown for market in West Honiara and peri-urban areas of Auki. It is mainly grown in the streams in the mountains where stretches are dammed using logs or rocks.

The production of watercress crops is limited by insect pests, mainly *P. xylostella,* that cause damage to the harvested leaves. One management strategy that is currently being tested is to grow the watercress on floating rafts, which are sunk periodically to drown the pests. In ball cabbage production, *P.xylostella* damages the developing leaves in early growth stages. Other insects such as *C. pavonana* and *S. litura* burrow into the young growing tips, damaging the developing head and making them unmarketable. The use of synthetic insecticides as a management strategy is neither appropriate or to be encouraged in the Solomon Islands. The farming practices presently utilize many natural resources such as mulch and compost. Pesticide supplies are limited and farmers have limited or no knowledge and skills about how to use them appropriately. The use

of exclusion row covers is an effective pest management practice that has been used to reduce caterpillar, whitefly, thrips and aphid numbers and crop damage in a variety of high value vegetable crops.

In this research, the approach to managing pests in these *Brassica* crops is centred on methods that are appropriate for farmers in the Solomon Islands. Practical methods that utilized inexpensive, locally available materials that could be applied with little technical supervision were important. The purpose of the study was to determine the efficacy and cost effectiveness of an easy to use technology that could help farmers manage troublesome pests on a high value crop. To this end, the research focused on methods that could exclude the insect pests for periods long enough for crops to develop and set marketable heads. Exclusion treatments that included a floating row cover and an insect net that could be placed over the growing crop were chosen for comparison. The use of floating row covers to regulate crop growth is a well established technology which is practiced in many countries, particularly in temperate climates where it promotes the retention of heat, which enhances plant growth and earlier yields. Floating row covers (Reemay® and Vispore®) provided effective frost protection and extended the growing season of tomatoes and cucumbers in Oregon, USA (Nelson et al. 1985). In field studies, the row covers increased the transplant success rate and increased soil temperatures which helped stimulate fruiting and increased yields (Nelson et al. 1985).

The use of floating row covers and pyriproxfen were shown to reduce fruit damage caused by silverleaf whitefly (*Bemisia tabaci*) on zucchini, resulting in an increased percentage of marketable fruit in Queensland (Qureshi et al. 2007). In that study, the floating row cover alone produced the highest marketable fruit for most of the sampling dates (Qureshi et al. 2007). Floating row covers combined with plastic mulch also helped control virus transmitting insect vectors on zucchini in Florida (Webb and Linda 1992). The row covers produced zucchini plants that were larger and more vigorous in size than uncovered plants which were infected with virus and stunted (Webb and Linda 1992). Virus disease incidence in cantaloupes was delayed due to row covers (Agribon-17) completely excluding insect vectors in Mexico (Orozco-Santos et al. 1995).

The financial aspects of utilizing these techniques have also been investigated. The economic benefits to growers and consumers of using insect netting in greenhouse tomato production to exclude whitefly vectors causing tomato yellow leaf curl (TYLC) was proved (Taylor *et al.* 2001).

This paper evaluates two types of row covers used in the management of cabbage *(Brassica oleracea var.* capitata*)* in the Solomon Islands.

MATERIALS AND METHODS

The exclusion row covers and insect net, which are not locally available, were sourced from Australian suppliers and delivered to the Solomon Islands. Materials used to make hoops to support the netting over the plots were sourced locally. Trials were conducted in three locations in the Solomon Islands - Busarata (Malaita), Henderson (Guadalcanal) and Tetere (Guadalcanal). Two of the locations (Henderson and Tetere) are in the lowlands, where ball cabbage can no longer be grown economically due to the constraint posed by insect pests, and a third location (Busarata) was in the highlands, where production exists. Two exclusion treatments (row covers) were compared with no exclusion (control) in field evaluations. The two exclusion treatments were;

MikroKlima® GrowCover (Veggie net) (Supplier: Veggie Patch, Australia) is woven polyethylene netting of uniform weave, with a density of 35g/m^2, 85% light penetration and 2 m width.

Evolution® Row Cover [Supplier: Gundaroo Tillers, Australia; Manufacturer: Kimberly Clark (Australia & New Zealand)] is made from lightweight High Density Polyethylene (HDPE) fleece fabric, with a density of 20g/m^2 and 2 m width.

Installation of exclusion treatments

Immediately after seedling transplanting, the exclusion nets were placed over the cabbage plots. The nets were supported by 20 mm polypipe hoops (each 1.8 m long). Lengths (≈50cm) of 16 mm iron bars were pushed into each end of the polypipe, and were then pushed into the ground to secure the hoops. Hoops spaced 1 m apart were placed over the beds to support the nets. The nets were cut to length with an extra 1 m for tying each end. The nets were placed over the hoops and secured with 'jumbo clips'. The ends of the net were tied and one edge was weighed down and buried. Exclusion net treatments that remained over the plots until harvest were periodically lifted on one side to weed, water, collect plant measurements and make observations.

Trial Plot details

All three trials had the same arrangement, using a latin square design with three replications. Plot sizes were approximately 1.5 m wide and 6 m long. The total size of each trial was also similar; 6.5 m wide (3 beds with a 1 m spacing between beds) and 20 m long (6 m plot lengths with a 1 m spacing between plots). Beds were hand cultivated and prepared with compost and manure approximately two weeks prior to transplanting cabbage seedlings which had been raised on farm nurseries.

Trial locations

Location 1: Busarata

The trial at Busarata, Malaita was established on 22nd April 2010. Busarata is located in the highlands 800 m above sea level and consists mainly of steep land that is loosely terraced. The soils are sandy loam (no soil analysis completed) with pH 7. In this location, carrots and onions had been grown previously on the site. Twenty seedlings were transplanted into each bed, in two rows (30 cm apart), 60cm spacing, after the nets had been put in place. Harvest was conducted on 9th August 2010.

Location 2: Henderson

The trial at Henderson, Guadalcanal was established on the 6th May 2010. Henderson is located 11 km east of Honiara, on the edge of the Guadalcanal plains. The land is low lying and periodically flooded by storm water runoff from the airport. The soils are clay loams and are quite low in nitrates (14 nitrate-N/ha) and have a pH of 6.5. In this location, rock melon had been previously grown on the site. Twenty seedlings were transplanted into each bed, in two rows (30 cm apart), 60 cm spacing, before the nets were placed over the hoops. Harvest was conducted on 19th July 2010.

Location 3: Tetere

The trial at Tetere, Guadalcanal was set up on the 9th August 2010 at Don Bosco Rural Training centre farm, 70 km from Honiara. The land is generally flat, with soils that are heavy clays, with a pH of 5.5 and very low nitrate levels (5.87 nitrate-N/ha). In this location, eggplant had been previously grown on the site. Twenty seedlings, sown on 6th July, were transplanted, in two rows (30 cm apart), 60 cm spacing. The seedlings were transplanted before the nets were placed over the hoops. No Evolution Film® row cover was included in this trial due to limited space available and its poor durability in the other trials.

Data collection

Different types of data were collected at three different stages of growth.

Growth stage 1 (0 – 10 weeks after transplanting) was prior to head formation. The data collected at this stage was: average number of leaves per plant, percentage leaf damaged by caterpillars, number of pests (physical counting of each species). This data was collected 2-3 times.

Growth stage 2 (11 weeks after transplanting) was at head formation stage. The data collected were the number of insects present and the head size (diameter in cm). This data was only collected once.

The number of leaves and size of head at stage 1 and 2 were used to determine the effect of the exclusion treatments on the plant growth characteristics.

Growth stage 3 (17.5 weeks) was at harvesting. The total numbers of harvestable heads were recorded, along with size of the head (cm), weight of head (g), quality of head (graded by participating farmer) and market price (SBD).

RESULTS AND DISCUSSION

Plant growth characteristics

Plant vgor - Number of leaves

There was no significant differences in the number of leaves produced under different nets at growth stage 1 ($F_{2,15}$=0.32, p=0.732) and growth stage 2 ($F_{2,15}$=0.08, p=0.926). Interaction between trial sites and exclusion net treatment was also not significant, which indicates that the effect of exclusion row covers across the trial was similar (in terms of plant growth). There was a significant difference in the number of leaves produced between sites at growth stage 1 ($F_{2,15}$=12.21, p<0.001) but not at growth stage 2 ($F_{2,15}$=0.15, p=0.862). However, the plants growing at Busarata under Evolution film® row covers developed heads later than the other two treatments.

Head Size

For practical reasons, head size at growth stage 2 was collected only at Henderson. The results indicated no significant difference between the treatments.

Henderson was the only location where a complete set of data was collected. Heads did not develop under Evolution Film® row covers (F) at Busarata and Tetere so pair-wise comparisons were conducted. Differences between Mikroklima® net (V) and no-net treatments for head size at harvest was not significant for means averaged over the three locations ($F_{1,15}$=0.10, p=0.758).

For Henderson, the head size from Mikroklima® net (V) is comparable to both Evolution Film® row cover (F) and no-net (N), but Evolution Film® row cover had a smaller head size than no-net.

There was however, a significant difference between head size across sites, with those under Mikroklima® net at Busarata significantly larger than at Tetere ($F_{2,13}$=8.31, p=0.005), but comparable with that at Henderson. Without net, Tetere had significantly smaller head size at harvest than the other two sites.

Crop Maturity

The harvest dates for treatments at each trial site, with the same planting date, were the same, so no differences in crop maturity were measured. Although head size of treatments was measured (above) there was no attribution to/with treatment.

Plant damage and pest presence

The level of pest presence was determined by the percentage (%) of damaged leaves (prior to head formation) and total number of pests observed (on the plants at Growth Stage 1 and 2). Damage to leaves was characterized by holes chewed by caterpillars. Observing and counting the number of caterpillars and other insects present determined pest presence.

Plant damage

The percentage of damaged leaves on plants in exclusion row cover treatments was significantly lower than plants in the control plots at all locations ($F_{2,15}$=4.04, p=0.039). At Tetere, the mean percent damage in the plots without net reached 96% compared to 47.5% under exclusion row cover / net (Fig 1). The extent of pest damage in one replication resulted in plants being completely destroyed during early growth stages. There was no significant difference between the two types of exclusion row cover / net for pest presence and the percentage of damaged leaves.

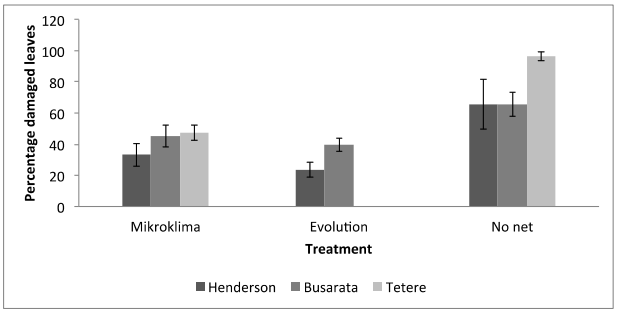

Figure 1. Percentage leaf damage observed prior to head formation (V=Mikroklima® net, F=Evolution Film® row cover, N= no exclusion row cover).

When damage at early stage (Plant Growth Stage 1) of development was compared with later stage (Plant Growth Stage 2), there was a slight increase in percentage of leaf damage under the exclusion row cover treatments, compared with no net, which increased by 24% (Table 1).

Table 1. Percentage leaf damage at Plant Growth Stages 1 and 2 (growth prior to head formation).

Plant Growth Stage	Net	Row Cover	No net cover
1	39.3	39.1	63.8
2	44.4	24.45	87.9

Pest presence

Exclusion row cover treatments were effective in preventing pest infestation at Growth Stages 1 and 2 in plants in Busarata and Tetere, but not in Henderson (Fig 2).

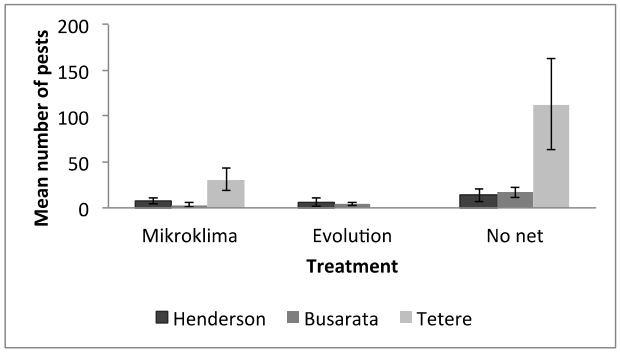

Figure 2. Mean number of pests observed 7 weeks after transplanting (V=Mikroklima® net, F=Evolution Film® row cover, N= no exclusion row cover).

Three main Lepidoptera pests were observed. At Henderson, *P. xylostella* had the highest populations in all three treatments (Fig. 3). Although *S. litura* had the lowest populations, the damage they caused had a bigger impact on yield. This was mainly due to their feeding behavior, targeting the heart of the cabbage, resulting in the head being destroyed.

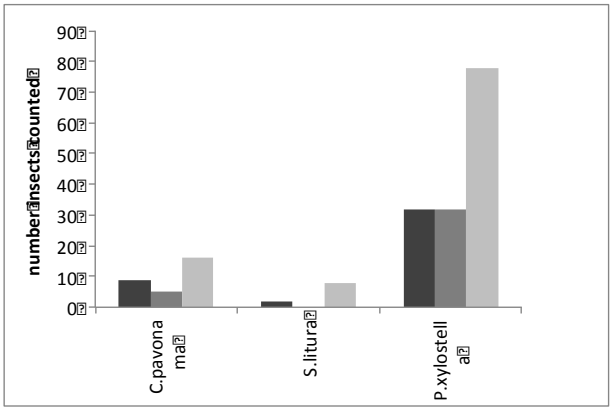

Figure 3. Henderson site - Total number of the three main Lepidoptera species observed in each treatment

Crocidolomia pavonana was present throughout all plant growth stages, and mainly fed on the leaves. *S. litura* started to appear at the head formation stage and caused the most damage, particularly in the no-net treatment. *P. xylostella* didn't appear on plants until later in the crop cycle.

A similar pattern was observed for *C. pavonana* and *P. xylostella* in Busarata, although the latter appeared earlier in the crop development. This may have been due to the close proximity of watercress acting as a host of *Brassica* pests.

At Tetere, the population of Lepidoptera species was much higher than the other two locations. *S litura* was the predominant species present, with only low numbers of *P. xylostella* observed. Thus, exclusion row cover treatments did not seem to provide any superior level of exclusion to particular types of pests.

Factors contributing to pest presence - Field operations & mechanical damage

The presence of insects under the row covers is attributed to farmers lifting the nets to weed and water, as well as during data collection. At Tetere, students regularly inspected the trial treatments, which resulted in the nets being disturbed more frequently than at the other trial sites. It was also possible that at all locations, insects laid eggs on the netting and some neonates then dropped onto the plants.

Insect presence under the Evolution Film® row cover net was also due to the net being damaged in the early stages of the trials, leaving small tears and holes in the cover. In Busarata, the row cover was snagged when setting up and in Henderson, the row cover in one plot was ripped.

Another contributing factor to the presence of pests inside the net was that all beds were prepared by hand and therefore the terrain was rough, making it difficult to seal securely.

Yield

Yield evaluations were determined by the average weight of heads at harvest, total number of heads harvested and number of heads marketable. In addition to weight, size was also considered as a yield factor as head size is the most important determinant of market price. The Henderson trial site was the only location where reliable actual market sales data was collected.

Figure 4 presents the mean cabbage head weight (g) at the three trial locations. The difference between Mikroklima® net (V) and no net (N) was significant for mean head weight across the three locations. *i.e*., head weight in plots under Mikroklima® (V) (6.19) was significantly bigger than those from no-net (N) (5.92). Under both Mikroklima® (V) and no-net (N), Busarata yielded significantly higher head weight at harvest (columnwise comparison (x, y). Analysis was performed based on values transformed to log scale.

There was a significant difference in the percent marketable heads between sites, ignoring treatments ($F_{2,13}$=13.10, p<0.001) and mildly significant between treatments ($F_{2,13}$=3.39, p=0.065). Percentage marketable yield was significantly lower in the no-net treatment (N) in Tetere than the other sites ($F_{2,5}$=9.76, p=0.019) while there was no significant difference in the marketable heads grown under Mikroklima® ($F_{2,6}$=2.45, p=0.167). At Henderson, 97% of the heads harvested from under the Evolution Film® row cover were considered marketable, compared to 57% for the Mikroklima® net and 30% for the no-net treatments. In Tetere, only 9.17% of the heads harvested from the no-net treatment were marketable. The quality of the seedlings at Busarata was far superior than the other two trials, which could have contributed to the higher yields in that location.

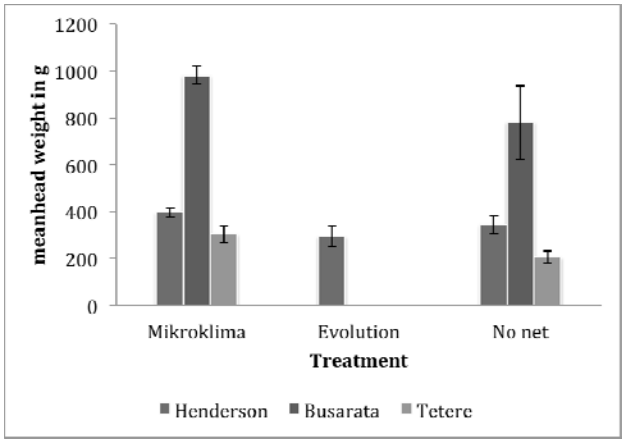

Figure 4. Yield (mean harvested head weight g) of ball cabbage

In Solomon Islands markets, marketability is not always directly related to high quality (*i.e*., no pest damage), but purely based on the farmer's opinion on what s/he can sell. Consumers are often content to buy heads with insect damage as there is rarely an alternative.

Economic evaluation of exclusion row covers

The cost evaluation was only calculated for the Mikroklima® net as the durability of the floating net was not acceptable.

Overall economical benefit

The net present value

The net present value of the insect netting is the present value of the benefit minus the present value of the cost of investment. The annual benefit is the cost saving, being the yield multiplied by the change in price due to the netting (Taylor et.al. 2001).

The cost of setting up Mikroklima® net for 6 beds of 10 m would in total cost SBD 1,610.92. On the assumption that the net will last for three years, the annual cost of net would be SBD 536.97.

There are many factors that affect the change in price in the market, which influence the approach to analyzing the data. Produce is generally sold in the wet markets by volume and prices are rarely influenced by the quality of the product. It would not be uncommon in the market for ball cabbage heads that are heavily damaged by pests such as *P. xylostella* to sell for the same price as "clean" heads.

The main effect that will directly influence price is the reduced size of heads due to damage whilst growing. The main reason for this is the perception that if produce has insect damage, the farmer would not have used pesticides and therefore it is safer for consumption. The real value of a product in relation to quality is only realized through direct markets, which were not accessed by farmers in this trial, except for Henderson. Fig. 5 shows the correlation between price and weight.

For the analysis of the net present value, the yield was calculated as marketable percentage based on a total expected yield of 150 heads. The price was calculated on the average expected price from actual yield for each treatment.

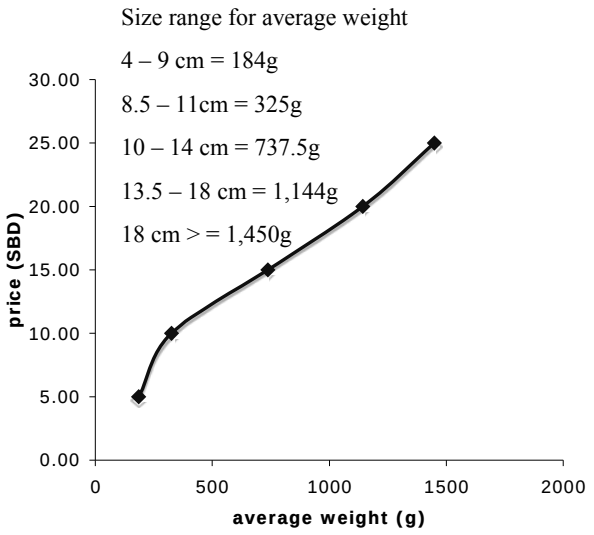

Figure 5. Price structure in relation to size and weight of ball cabbage head during trial period

Table 2 shows the data used to calculate the present value of benefit based on 2 crops per year

Table 2 Calculation of present value of benefit

Location		%	Yield 100m^2	Price/h (SBD)	Price diff.	Benefit per an
Henderson	V	57	85.50	14.56	8.19	1,400.49
	N	30	45.00	6.38		
Busarata	V	91	136.50	15.98	7.05	1,924.65
	N	84	126.00	8.93		
Tetere	V	51.9	77.85	6.23	1.23	191.5
	N	9.17	13.76	5.00		
				Mean	**5.49**	**1,172.2**

Based on the prices of cabbages at the time of the trial, the net present value for the three sites is 863.52, 1,387.68 and -345.46 for Henderson, Busarata and Tetere, respectively.

General observations

Exclusion net characteristics and properties

The floating row cover, although highly effective at excluding pests, was not very durable in the environment where it was tested. During the trial, the floating row covers at both Henderson and Busarata were damaged, resulting in the decision to exclude it from the final trial in Tetere. Deterioration of row cover materials due to tearing was also reported by Webb and Linda (1992). This aspect potentially limits this row cover to one use and may affect the capacity to exclude insects. Lower costs for this type of material may be a compensating factor.

Both types of nets resulted in an increase in ambient temperature under the netting treatment, which was noticeably higher in the floating net. During the trial it was observed that both nets had an effect on the micro-climate around the plants - this included air temperature and relative humidity. The effect was particularly noticeable in Busarata, where the air temperature is generally much lower than the other two locations.

The methods of placement of the exclusion nets over the plants and beds provided access for various tasks. It was possible to lift the row cover and insect netting (on one or two sides) to carry out weeding, general observation of plants, inspection of plants for pest and disease incidence and check for maturity.

CONCLUSION

The results of this trial have demonstrated that exclusion row covers have the potential to improve the quality of ball cabbage production. It would be cost effective for farmers to invest in row cover technology as cost recovery would be possible even after two years. Farmers would also potentially have access to high-value, direct markets that would further increase their income. This will also subsequently, reduce the need for high-value markets to import to meet their demand for high quality heads. In addition, production can extend into previous areas abandoned due to high insect pressure with this technology. The materials used in the trial however, could be substituted with lower cost alternatives and evaluated for other crops that could be incorporated into a crop rotation reducing the need to remove the structures between crops.

Acknowledgements

The author would like to thank the staff at AVRDC-The World Vegetable Center - Solomon Islands office, Doreen Suimae and Medlyn Dick; Ministry of Agriculture and Livestock, Don Bosco Rural training Center and the farmers in Busarata and Henderson for assisting with setting and conducting the trials; Dolores R. Ledesma from AVRDC-The World Vegetable Center – HQ for conducting the statistical analysis on the data; Michael Day from DEEDI, Australia for assisting with the statistical analysis interpretation and editing.

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Biological control of diamondback moth on cruciferous crops in Myanmar

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ABSTRACT
Diamondback moth (DBM), *Plutella xylostella* is a serious pest on cruciferous crops worldwide, including Myanmar. The objective of this study was to control the population of DBM, and to increase the natural enemies. Laboratory experiments were conducted to choose the most suitable diet among cabbage, cauliflower, broccoli and artificial diet for DBM mass-rearing. Another experiment was conducted to choose the best medium for pupation using aluminum foil, tissue paper and normal paper. Parasitism rates of DBM were estimated with a larval parasitoid (*Cotesia plutellae*) and two pupal parasitoids (*Diadromus collaris* and *Macromalon orientale*). Mean percentage of DBM adult emergence was 73.30% on cabbage as a host plant, followed by 70.00% on cauliflower, 60.00% on broccoli and 53.30% on artificial diet. Hence, DBM development was better on cabbage compared to cauliflower, broccoli and artificial diet. Mean Percentage of DBM pupation on various media was 86.70% for aluminum foil, 83.30% for tissue paper and 73.30% for normal paper. Mean percentage of DBM parasitism rates were 73.30% by C. *plutellae*, 63.30% for D. *collaris* and 56.70% for M. *orientale*. Hence, parasitism rate of DBM larvae by the larval parasitoid (C. *plutellae*) was higher than the pupal parasitoids (*D. collaris* and *M. orientale*). The results on DBM parasitism by various parasitoids encourage the use of natural enemies to control DBM on cruciferous crops in Myanmar.

Keywords
Diamondback Moth, *Costesia plutellae,Diadromus collaris, Macromalon orientale*,host plants

INTRODUCTION
Diamondback Moth (DBM), *Plutella xylustella* is a serious pest on cruciferous crops worldwide, including Myanmar (Talekar & Shelton, 1993; Morris and Waterhouse, 2001). To control DBM, farmers use large quantities of insecticides. Intensive and continued use of insecticides resulted in DBM developing resistance to insecticides in several regions. The objectives of the study are to control the population of DBM, to increase the population of natural enemies and thus reducing the use of chemical pesticide in cruciferous fields.

MATERIALS AND METHODS
The studies included tests of DBM rearing on natural host plants and artificial diet, DBM pupation on various media and parasitism rates of DBM by larval and pupal parasitoids under laboratory conditions during 2009-2010.

Diamondback moth rearing on natural host plants and artificial diet

Second instar larvae of DBM were fed on natural host plants (cabbage, cauliflower and broccoli) as well as artificial diet until pupation. Ten larvae were used per replication, and there were three replications for each diet. Upon adult emergence, the mean percent eclosion was calculated for each treatment.

Identifying the best medium for DBM pupation

Aluminum foil, tissue paper and normal paper, each in a dimension of 12-cm X 8-cm were tested for their suitability as a medium for DBM pupation. Ten fourth-instar DBM larvae were fed on cabbage. When the larvae were about to pupate, they were transferred to aluminum foil, tissue paper and normal paper for pupation. For each treatment, three replications were maintained, and the mean percent pupation was calculated.

Parasitism rate of DBM larvae and pupae

Ten second-instar larvae of DBM were exposed to the larval parasitoid, *Cotesia plutellae* for about 24-h. The parasitized DBM larvae were reared on cabbage plants until the DBM larvae or the parasitoid attained pupation. Similarly, ten DBM pupae each were exposed to the pupal parasitoids, *Diadromuscollaris* and *Macromalon orientale*. The parasitized pupae were maintained under laboratory conditions until the DBM adults or the parasitoid adults emerged out. For each treatment, three replications were maintained.

STATISTICAL ANALYSIS

The data collected were statistically analyzed using IRRI stat. Treatment means were compared using the LSD technique at 0.05 and 0.01 probability level.

RESULTS AND DISCUSSION

Diamondback moth rearing on natural host plants and artificial diet

Mean percentage of DBM adult emergence was 73.30% on cabbage, followed by cauliflower (70.70%), broccoli (60.00%) and artificial diet (53.30%) (Table 1). DBM rearing was better performing on cabbage and cauliflower than broccoli and artificial diet. This has been proven in several earlier studies. For example, DBM exhibited a marked preference for cauliflower and cabbage among several crucifers, probably due to the fact that both plants possess fleshy and succulent leaves, which provided olfactory and gustatory stimuli for successful host selection and development (Chelliah and Srinivasan, 1986).

Identifying the best medium for DBM pupation

Mean percentage of DBM pupation was 86.70% on aluminum foil, 83.30% on tissue paper and 73.30% on normal paper (Table 2). Hence, DBM pupation was better on aluminum foil and tissue paper than normal paper.

Parasitism rate of DBM larvae and pupae

Mean percentage parasitism of DBM larvae was 73.30% by the larval parasitoid, *Cotesia plutellae*. The mean pupal parasitism rates were 63.30% for *D. collaris* and 56.70% for *M. orientale* (Table 3). In general, the larval parasitoid was more efficient than the pupal parasitoids. Among the pupal parasitoids, *D. collaris* performed better than *M. orientale*. It has already been shown that the release of irradiated male DBM and the release of the parasitoid, *C. plutellae*, can reduce the seasonal increase of DBM populations in cabbage in Myanmar (Maung 2002). *C. plutellae* is the dominant larval parasitoid of DBM in Thailand. Parasitism has been reported as high as 88%. It has been found during every month of the year in parts of northern Thailand where crucifer crops are grown year-round. It has even been found in some fields in the North where harsh organophosphate pesticides were being used, which suggests that some populations of *C. plutellae* may have become resistant to pesticides used in certain areas. Parasitism of host pupae by D. collaris ranged from 9 to 31% in Thailand. *M. orientale* has been found only in Chiang Mai province of Thailand, where the average DBM parasitism by this parasitoid was only 0.5-6%. *M. orientale* has never been mass reared and released in Thailand, although it is considered an important DBM parasitoid in the Assam region of India (Rowell et al., 2005). Thus, our results also confirmed the fact that *C. plutellae* performed better, which was followed by *D. collaris* and *M. orientale*.

Acknowledgements

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TABLES

Table1. Diamondback moth rearing on natural host plants and artificial diet, 2010

No. adults	Treatment	DBM
1.	Cabbage	7.3
2.	Cauliflower	7.0
3.	Brocoli	6.0
4.	Artificial diet	5.3
	LSD at 5%	1.2*

*; $0.05 \leq P \leq 0.01$

Table 2. Identifying the best medium for DBM pupation

No.	Treatment	Pupation
1.	Aluminum foil	8.67
2.	Tissue paper	8.33
3.	Normal paper	7.33
	LSD at 5%	1.51^{ns}

ns; not significant

Table 3. Parasitism rate of DBM larvae and pupae

No.	Treatment	Parasitism
1.	*Cotesia plutellae*	7.3
2.	*Diadromus collaris*	6.33
3.	*Macromalon orientale*	5.67
	LSD at 5%	1.51^{ns}

ns; not significant

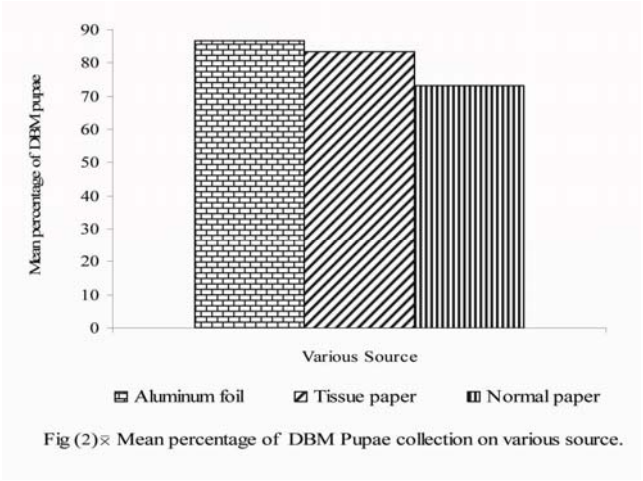

Fig (2)※ Mean percentage of DBM Pupae collection on various source.

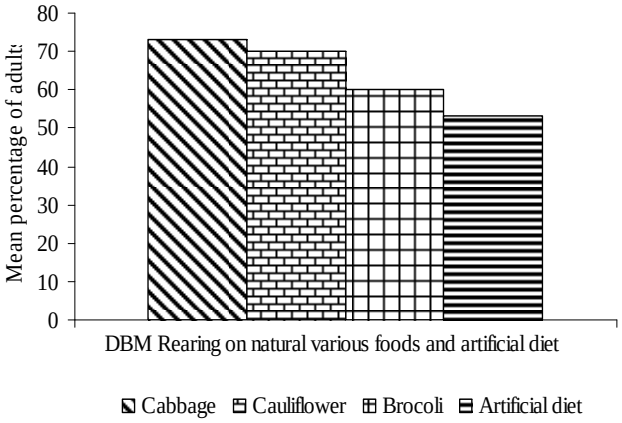

Fig (1)□Mean Percentage of DBM rearing development on natural various foods and artificial diet (2nd instar larve to adults) (February,

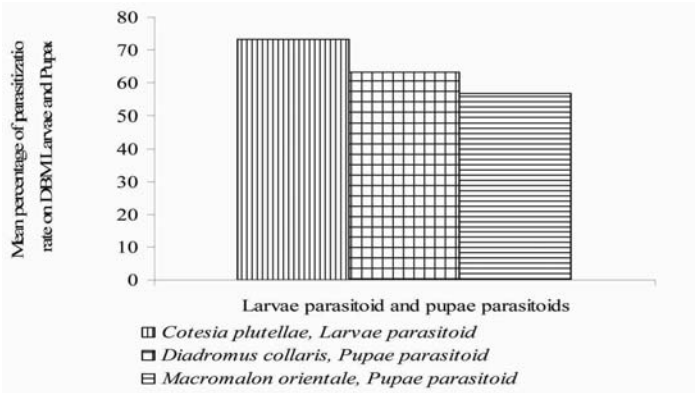

Fig (3)※ Mean percentage of parasitization rate on DBM Larvae and Pupae.

Myco-Jaal: a novel formulation of *Beauveria bassiana* for managing diamondback moth (*Plutella xylostella*) in subtropical and tropical crucifer production systems

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ABSTRACT
Myco-Jaal, in a mixture of emulsified liquid carrier and additives, has aerial conidia of *B. bassiana* specific to DBM, Diamondback moth, (*Plutella xylostella*) (L.), a major pest of crucifer in subtropical and tropical crucifer production. The novel formulation improved conidial viability thus enhancing the shelf life up to one year under normal conditions (< 40^{o}C and) and two years in refrigerated conditions. The environmental persistence was recorded to be higher (>8 days) than the other fellow formulation, aqueous and wettable powder under field conditions. Multilocation trials of Myco-Jaal @5x10^{12} conidia in 250-300 liters of spray suspension per acre (1acre=0.405ha) using a high volume sprayer under different agro-climatic conditions in India shows reduction of DBM population (62.50-70.00%) after a fortnight. Thus 500 ml of Myco-Jaal per acre per application, with 3-4 application per crop cycle from head forming stage onward was found to be an effective dosage for DBM management. Although Myco-Jaal took some initial time (5-8 days) to establish the significant infection in DBM populations, it showed promising prolonged impact in suppressing DBM populations and even showed a high cost-benefit ratio over farmers' practices with high inputs of commonly used pesticides like Avaunt (Indoxicarb-15.5%SC), Rocket (Cypermethrin 4.0%+Profenfos 40%), and Spinosad 2.5%SC, which are used alternatively at weekly intervals at the recommended dosage of 0.5ml/L, 2-3ml/L and 2ml/L respectively. Myco-Jaal was found to be compatible even when integrated with other biorational pesticides (Dipel, Spinosad) and chemical pesticides (Avaunt, Rocket) in developing a package for cabbage growers. In addition, Myco-Jaal has shown good control over sucking pests like spider mites, aphids and thrips, many of which sometimes attack crucifers. The product has successfully completed the stringent rule related to efficacy, biosafety by the Central Insecticide Board and Registration Committee, Government of India and is now commercially available throughout India and in South Asia for sustainable and eco-friendly management of DBM and other sucking pests of crucifers and a range of other vegetable crops.

Keywords
Beauveria bassiana, Suspension concentrate formulation, diamondback moth, Myco-Jaal

INTRODUCTION
Growing awareness about harmful effects of agrochemicals and demand for organically grown agricultural produce have resulted in a shift of focus from chemical to bio-pesticides for management of agricultural crop pests. There has been a global upsurge in interest in the development of microbial pesticides, particularly entomopathogenic fungi, due to ease of in-vitro production techniques, and breakthroughs in strain selection and formulation technology. But the potential of these organisms is yet to be exploited in Indian agriculture with improved techniques due to inconsistent performance of locally available formulations. Considering this Bio-Control Research Laboratories (BCRL), a pioneer in commercial production of biological control agents in India, initiated work on development of improved formulations of myco-pesticides in 2001. This work was inspired by the successful development of "Green Muscle," a ULV formulation of *Metarhizium anisopliae* under the LUBILOSA Project which reported >90% control of desert locust in South Africa under high temperature and low humid conditions (Bateman 1997).

The diamondback moth (DBM), *Plutella xylostella* is considered to be one of the most destructive lepidopteran pests on cruciferous vegetables worldwide (Talekar and Shelton 1993) and reported to cause yield loss from 31% (Abraham and Padmanabhan 1986) to 100% (Cardleron and Hare 1986). DBM has become problematic because in several parts of the world it has developed resistance to most commercial insecticides, including some growth regulators and toxins of microbial insecticides, *Bacillus thuringiensis* (Perez et al. 1997). The alarming rate of increase in usage of different chemical pesticides in cabbage cultivation has led to the search for some eco-friendly, cost-effective alternatives. Mycopathogens have emerged as potential agents among insect pathogens, which have unique modes of action not easily amenable for insects to develop resistance. In particular, broad spectrum pathogen *Beauveria bassiana* has been reported to invade and cause disease in lepidopterans including *P. xylostella* (Robert and Marchal 1980). An oil-based commercial formulation of *Beauveria bassiana*, Mycotrol from Mycotech Corp. of USA, has proven to be an effective alternative for the management of DBM on cabbage (Shelton et al. 1998). According to

the market survey, available biopesticides based on wettable powder compared to the commonly used chemicals are not very promising due to their inconsistent field performance. Conidial inability to survive under adverse environmental conditions and ultraviolet radiation can be overcome by developing suitable formulations (Jaronski 1997, Bateman et al. 1993) prompted BCRL to initiate work from the selection of the most virulent fungal isolate of *Beauveria bassiana* for DBM, standardization of mass production and special emphasis on effective formulation (Ghosh et al. 2003) These efforts resulted in an oil-based emulsified suspension concentrate formulation of *Beauveria bassiana* with trade name Myco-Jaal® 10% SC ($1x10^{10}$conidia/ml), which was registered with the Central Insecticide Board and Registration Committee (CIB & RC) of India as the first oil-based formulation of mycopesticide in the country.

Importance of DBM as a target pest

India is an agrarian country and the second largest producer of vegetables in the world, after China with an annual production of 81 million t from 5.12 million hectares of land (Karanth 2002). Cruciferous vegetables, especially cabbage and cauliflower, are important cole crops in India, grown on about 0.438 million hectares, producing about 6.335 million t per annum (Mohan and Gujar 2003). The main constraint to production is the diamondback moth, which causes an average of 52% losses to marketable yields (Kumar et al. 1983) and sometimes even can destroy the entire crop (Gujar, 1999). The destructive stage larvae of the diamondback moth cause intense damage to crucifer crops such as cabbage, cauliflower, broccoli, Chinese cabbage, Chinese kale, and others. Costs to control diamondback moth (primarily pesticide costs) were estimated to be US $1 billion per year worldwide (Talekar 1992, Talekar and Shelton 1993). In Asia, DBM has become resistant to nearly every class of insecticide due to continuous use of new and effective insecticides. This overuse also causes health problems for farmers applying the pesticides, along with contamination of soil and water, resulting in excessive residues on vegetables. In addition, natural enemies of diamondback moth and other pests are killed because of over-reliance on pesticides (Rowell, 2000).

Indiscriminate use of insecticides, multiple generations of diamondback moth per year, and the year-round availability of host crops also have contributed to the development of resistance in this pest to almost all kinds of insecticides (Sivapragasam et al. 1996, Ferre and Van Rie 2002) with cross-resistance and multiple resistance to many insecticides (Joia et al. 1994), increasing number of sprays, and rising costs of cultivation. Resistance is estimated to cost US $8.8 million in Karnataka alone (Kumar 1995). Despite availability of less toxic and eco-friendly newer molecules in the market, farmers still prefer and use broad-spectrum organophosphates, pyrethroids, organochlorines and other conventional insecticides—to most of which, diamondback moth has developed resistance. However, price of a pesticide was found to be a major determinant for preference. Among the biorationals, *Bacillus thuringiensis* (Bt) is a major biocide, but DBM already has been reported to have developed resistance to Bt formulations Dipel and Thuricide in different parts of the country. Therefore, one has to be very cautious in using Bt products for the control of DBM (Krishnamoorthy et al. 2000). There is definite scope for transgenic Bt cabbage seeds as alternatives to pesticides in minimizing pest attack and cultivation costs, but the impact on the environment must be studied.

Biological control, which involves the introduction, augmentation and conservation of natural enemies, has already proven itself to be a valuable weapon in pest control of a number of crops. Several natural enemies of DBM like *Trichogrammatoidea bactrae, Diadegma fenestrale, Cotesia plutellae* were reported but their role in the control of DBM is not clearly quantified. A granulosis virus isolated from *P. xylostella* (PxGV) was found capable of curtailing the development of resistance by interacting and synergizing with the insecticides (Rabindra et al. 1996). Laboratory experiments with a LC_{25} dose of abamectin 1.9% EC, indoxycarb 14.5%SC, cartaphydrochloride 25% WP and fipronil 5% SC on *P. xylostella* exposed to PxGV resulted in supplemental synergism. A nuclear polyhedrosis virus obtained from *P. xylostella* (PxNPV) also was evaluated under field conditions and when applied at $1.7x10^{8}$ POB/ml mixed with Indan ink as a sunlight protectant gave the greatest reduction in insect population (Padmavathamma and Veeresh 1995) but its in-vivo production is a big limitation for commercial development. The pathogenicity of different fungal bioagents such as *B. bassiana*, *Paecilomyces fumosoroseus*, *Verticillium lecanii* and *Metarrhizium anisopliae* have been reported against *P. xylostella* (Gopalakrishnan 1989; Kennedy et al. 2001) and their potential has been demonstrated in an ideal high humid crop ecosystem like cabbage and cauliflower but there has been little effort to develop a commercial product in a effective formulation.

Most biotechnology research and development focuses on major agricultural crops but large potential markets exist for biotech vegetables, especially in developing countries where the economic, health, and environmental benefits could be huge. Losses due to diamondback moth in tropical and subtropical crucifers can reach up to 90% if not sprayed and 35% even if sprayed. Massive applications of chemical insecticides are required, posing serious health risks to millions of smallholder farmers who grow these crops, as well as to the consumer. Considering the present status of DBM, the existing control measures, and commercial feasibility, we aimed to develop a mycopesticide as an effective tool for the integrated pest management of DBM.

Mode of infection of *Beauveria bassiana*

The only infectious stage of *B. bassiana* is the haploid

asexual conidium. The conidia adhere to the cuticle of insects, producing a germ tube and penetrating into the haemocoel where the hyphae eventually kills the host. After death, if conditions are warm and moist, the fungus grows back out through the cuticle, forming chains of new white conidia. The conidia do not disperse by wind or soil and therefore each killed cadaver forms a pool of conidia ready to attack and infect a suitable insect when it moves into the zone. Similarly, man can intervene and provide dispersal by means of spray or other formulations to develop the fungus into a mycopesticide. Many of its isolates are specific to particular groups while other isolates have a more general host range. Isolates grow best between 20-30 $^{\circ}$C though some are thermophobic growing at 10 $^{\circ}$C and thermophillic growing at 35 $^{\circ}$C or above. The main reservoir of conidia is the soil, where conidia can remain alive in dormant stage for many years without any saprophytic growth. Majority of safety testing has shown that the fungus is not harmful to life forms other than insects (Ravankar et al. 1999).

DEVELOPMENT OF MYCO-JAAL

There is a clear and demonstrated potential for using fungi successfully in insect pest management. Fungal efficacy has been shown in a diverse range of environments, including ones that may be thought of as being hostile to fungal infection processes due to high temperature extremes and conditions of low humidity. Successful introduction of fungal biopesticides requires identification of virulent isolates, improved and economical methods for large-scale production of viable propagules, formulations with good storage stability, high level of quality control, suitable delivery mechanisms and a large stable market.

Obtaining suitable candidate of fungal strain for product development

Considering the need for continuous intervention with different chemical pesticides throughout the crop cycle, diamondback moth was selected as the target pest. *B. bassiana* was isolated from a naturally infected DBM larva and further it was selected based on laboratory bio-assays involving 8 isolates of *B. bassiana*, 4 isolates of *M. anisopliae* and 2 isolates of *Paecilomyces fumosoroseus.* Its virulence was raised subsequently to 80% from the initial observed mortality of 35-40% by repeated exposure, isolation, and purification over 50 generations on lab-reared DBM larvae. Studies on environmental competence, biological, and ecological fitness, suitability for mass-production and genetic stability of the selected isolate were also carried out, which was essential to understand what extent of strain is suited for field conditions and commercialization.

Mass production

A well defined production system with in-built quality control is essential for the commercialization of fungal biocontrol agents (Wraight et al. 2001). We developed in-house facilities to mass produce and preserve large quantities of stable, virulent inoculum of consistent quality in a cost-effective manner. Since aerial conidia are required for preparation of oil-based formulations, a diphasic process, involving liquid submerged and solid substrate fermentation, was standardized for mass production. Due to non-availability of required equipment locally, machines were designed and fabricated at BCRL for bringing down moisture level of the conidial substrate from 70 to 15%, harvesting of conidial powder, and drying (<5% moisture content) of spores without affecting their viability. The indigenously designed spore harvester has the capability to handle 12 kg of substrate per hour and harvest 50-60 gm of pure conidial powder (1-5x10^{12} conidia/ gm) per kg. The harvested spores were found to remain viable for up to one year at room temperature and over two years under refrigerated conditions, validating the process and equipment.

Formulation and delivery

Formulation plays an important role for improved stability of fungi in storage, efficacy and persistence in the field (Goettel et al. 2000).Theoretically, preparation of an oil-based formulation involves suspension of conidial powder in a liquid phase of oil and effective combination of emulsifier, dispersing and suspension agents. Proper drying and particle size of the conidial powder, viscosity, and purity of the oil, properties of the surfactant, hydrophilic–lipophilic balance (HLB), selection of suitable dispersing agent, etc., were found to play a crucial role. The various problems encountered related to the final product included colloidal stability, homogeneity, control of sedimentation and prevention of caking of the formulated product, precipitation and flocculation rate of the suspension after tank mixing, phytotoxicity, and variable results during field trials. These issues were overcome after repeated laboratory and field studies carried out over a period of two years; based on the experience, standard operating procedures were prepared and protocols developed for regular assessment of quality, both of the process and the final product. The production process was monitored by assigning unique lot and batch numbers based on SDA slope subculture and the parameters monitored included contamination of substrate and production area, virulence of spores by laboratory bio-assays, etc. Similarly, quality parameters of the conidial powder used for preparation of the final product were assessed for contamination, moisture content, conidial number, viability, virulence and particle size distribution, which play a significant role in stability and field efficacy of the formulation.

In environmental persistence studies, a prolonged conidial viability was recorded in oil based SC formulation *B. bassiana* (nearly 10 days) compared with wettable powder (WP) formulation with >5 days persistence (Ghosh et al. 2003). In shelf-life studies, the conidial viability in Myco-Jaal was recorded more than one year compared with the WP formulation, showing > 6 months shelf life.

Efficacy of Myco-Jaal® against DBM

Lab efficacy

In bioassay study, LC 50 value of Myco-Jaal was recorded to be 0.6ml/l (6x 10^6conidia/ml) of water by leaf dip method under laboratory conditions against early 3rd instar larvae of diamondback moth (DBM) at $25+1^0$ C and 70-80% R.H (Ghosh et al. 2007).There was no significant difference when *B. bassiana* was treated on 3rd instar of DBM larvae in different form of formulation, aqueous, WP and SC under laboratory conditions but under caged field condition SC formulation performed significantly better than other formulation in terms of larval mortality (Ghosh et al. 2003). The additives and oil carrier of Myco-Jaal formulation definitely improved the conidial persistence and virulence by protecting the conidia from dessication and lethal effect of UV radiation under field conditions. The time taken for mortality was found to be comparatively less in Myco-Jaal formulation than WP, due to the activity of oil, which increases the adhesion and penetration of infective conidia through the insect cuticle for its lypophilic nature of both the cuticle and conidial surface (Wright and Carrutherus 1999).

Field efficacy

Field efficacy of Myco-Jaal was evaluated by conducting continuous multilocation trials under farmers' field conditions for more than 5 years before the product was made commercially available in India. Under farmers' field conditions, application of Myco-Jaal (@2 ml/litre) was found to reduce DBM population by 55.10% over control, up to 25 days after treatment, which was on par with Indixicarb-14.5% SC alone (60.00%), Myco-Jaal® in combination with Indoxicarb-14.5% SC (62.63%), and farmer's practices (61.62%), using a combination of different chemicals to prevent the development of resistance in DBM population. However, the highest yield was recorded in the treatment involving combined application of Myco-Jaal and Indoxicarb-14.5% SC with an increase of 6.8t/ha over control (Table 1). This was followed by Indoxicarb-14.5% SC (54.88 t/ha), farmer's practice (54.40 t/ha), Myco-Jaal (52.57 t/ha) and control (48.96 t/ha). The yield increase over control in Myco-Jaal® treatment was 3.6 t/ha. However, the highest cost-benefit ratio was obtained with Myco-Jaal 5.41, followed by Myco-Jaal in combination with Indoxicarb-14.5% SC (4.23), Indoxicarb-14.5% SC (3.84), and farmer's practice (2.60) (Ghosh 2007). Myco-Jaal is recommended to apply as a spray suspension in water at 5×10^{12} conidia in 250–300 l of spray suspension/acre (1 acre = 0.405 ha) using a high volume sprayer; thus 500 ml Myco-Jaal® will treat about an acre per application, with 3–4 applications per crop cycle. The product is compatible with synthetic chemical insecticides.

Registration

The process of registration of the country's first oil-based mycopesticide posed a number of hurdles, starting with generation of bio-efficacy data, both under laboratory and farmers' field conditions. Thus the standard procedure adopted for laboratory evaluation of wettable powder formulation was not found suitable for determining laboratory bio-efficacy and addition of ionic surfactant, which is a regular practice for field application of pesticides, was found to reduce product efficacy. The unique nature of the formulation caused delay in registration due to deficiencies raised on ingredients of the formulation. Central Insecticides Board and Registration Committee, Government of India, has granted registration for Myco-Jaal as a suspension concentrate formulation of *B. bassiana*. The product is loaded 1 x 1010 conidia with LC50 value of 6 x 106 conidia/ml of water suspension, as determined by bio-assays using 3rd instar larvae of DBM. Application of Myco-Jaal, 3-4 times during the crop cycle, starting with head formation stage, was found to be effective in suppressing DBM populations under farmers' field conditions. The bio-pesticide also was found to be compatible with synthetic chemical insecticides and combined application was found to provide better results when compared to sole application of either, indicating the potential of Myco-Jaal in bringing about a reduction in pesticide application, if adopted on a large scale.

Potential of Myco-Jaal on other economic pests

Growing awareness and market demand of organically grown agro-products in both local and export markets has opened an avenue for effective bio-insecticide formulations. Besides DBM, Myco-Jaal also has been found to be effective against various economically important pests like red spider mites (*Tetranychus urticae*), Cabbage aphids (*Brevicoryne brassicae*), thrips (*Thrips tabaci)*, and Tea mosquito bugs (*Helopeltis theivora*). The unique oil based properties of Myco-Jaal formulation have increased its market potential for other crops, such as tea, paddy, coffee, sugarcane, etc.

Safety aspect of Myco-Jaal

The safety studies were carried out at the International Institute of Biotechnology and Toxicology (IIBAT), Chennai, India against different vertebrates and invertebrates. Myco-Jaal 10% SC formulation of *B. bassiana* was found to be non-toxic and non-virulent against Swiss albino mice, New Zealand rabbit and albino rabbit with a maximum challenging dose of 5 ml containing 1x 10^{10} spore/ml. In eco-toxicological studies Myco-Jaal was found to be non-toxic and non-virulent against chicken, the LC 50 for *Labeo rohita* was considered as greater than 100 (1.3 x 10^9) mg/ml, the LD_{50} against *Apis carana indica* was recorded 2390.26

Table 1. Efficacy of Myco-Jaal on yield and CB ratio of cabbage cultivation

Treatments	Yield/plant (kg)	Yield/ha (t)	Additional		Input for DBM control (Rs.)	CB Ratio
			Yield (t/ha)	Return (Rs.)		
Myco-Jaal 10% SC	$1.32^{ab} \pm 0.04$	$52.57^{b} \pm 2.72$	3.61	10830	2000	**5.41**
Indoxicarb-14.5% SC	$1.39^{b} \pm 0.04$	$54.88^{b} \pm 2.72$	5.92	17760	4625	3.84
Myco-Jaal + Indoxicarb	$1.39^{b} \pm 0.04$	$55.76^{b} \pm 1.59$	6.8	20400	4312.5	4.73
Farmer's Practice	$1.36^{b} \pm 0.04$	$54.40^{b} \pm 1.70$	5.44	16360	6279.4	2.6
Control	$1.20^{a} \pm 0.05$	$48.96^{a} \pm 2.13$				
F-value	*	*				
CD at 5%	***0.12***	***3.33***				

microgram/bee when compared with standard check of endosulfan technical (LD_{50}= 1.09 microgram/bee) at 24 hours after dosing. Test result of toxicological studies of Myco-Jaal against early 3rd instar larvae of silkworm, *Bombyx mori*, showed no mortality even at a highest concentration of 1.60%v/v by leaf dip method and the LC_{50} of Myco-Jaal was found to > 1000 mg/kg dry weight of artificial soil at 14 days after treatments indicates non-toxicity to earthworm, *Eisenia fetida.* The microbial active ingredient, *B. bassiana* is a naturally occurring ubiquitous fungus, which is found in soils. It is not known to proliferate in aquatic habitats. Based on its low toxicity potential, it is not likely to pose an undue hazard to the environment.

Although Myco-Jaal has been developed to control DBM, it has enormous potential for other pests in different crop systems. Further research by extension services can confirm this prospect. Myco-Jaal is definitely an improved mycopesticide formulation that can be substituted for chemicals in a need-based application, or it can be used in combination with sub-dosage of chemicals to reduce chemical applications.

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Susceptibility of diamondback moth and cabbage head caterpillar to *Bacillus thuringiensis* (Bt) δ-endotoxins on vegetable brassicas in India

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ABSTRACT
The baseline susceptibility of diamondback moth (DBM), *Plutella xylostella,* from Gujarat (Anand), Karnataka (Bangalore) and West Bengal (Bolpur), and cabbage head caterpillar (CHC), *Crocidolomia binotalis* in Karnataka to *Bacillus thuringiensis* (Bt) toxins Cry1Ac, Cry1Ba2 and Cry1Ca4 was assessed. In most cases, the susceptibility of DBM to Cry1Ac was similar or slightly higher (mean LC_{50} ranging between 0.06 and 0.10 ppm) than Cry1Ba2 (mean LC_{50} 0.08-0.09 ppm) and Cry1Ca4 (mean LC_{50} 0.06-0.09 ppm), except Bolpur population to Cry1Ba2. The susceptibility of DBM to Cry1Ba2 and Cry1Ca4 was almost at similar levels. CHC was highly susceptible to all Cry toxins compared to DBM with LC_{50} values of 0.01, 0.05 and 0.04 ppm for Cry1Ac, Cry1Ba2 and Cry1Ca4 toxins, respectively. Because the susceptibility of DBM and CHC from Anand, Bangalore and Bolpur to the tested Cry toxins is higher than the previously reported values from other parts of India, it is possible that these insect populations can be controlled using commercial Bt formulations containing any of these Cry toxins, if available.

Keywords
Plutella xylostella, Crocidolomia binotalis, baseline susceptibility, *Bacillus thuringiensis* toxins

INTRODUCTION
Vegetable brassicas are one of the most important groups of vegetables in India, where they are grown over an area of 659,000 ha (FAO, 2009). Diamondback moth (DBM), *Plutella xylostella* L. (Lepidoptera: Plutellidae) is the most destructive insect pest of vegetable brassicas in India, sometimes causing almost 100% crop loss. In addition, cabbage head caterpillar (CHC), *Crocidolomia binotalis* Zeller (Lepidoptera: Pyralidae) may also infest the crop in different regions over different seasons (Srinivasan and Krishna Moorthy 1992; Tufail et al. 2009). The annual cost for managing DBM alone is estimated to be US$168 million in India (Sandur 2004). Only very few parasitoids such as *Trichogrammatoidea bactrae* Nagaraja (Hymenoptera: Trichogrammatidae) and *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae) have been exploited for the control of DBM. Although *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae), an imported Taiwan strain, was introduced in India, it did not establish in Indian lowlands where the temperature most of the year was above 35ºC during summer months (Krishnamoorthy 2002). Hence pesticides still account for about 38% of the total input requirements for major vegetable brassicas in parts of India (Shetty 2004). Due to extensive and inappropriate pesticide use, DBM has developed resistance to almost every class of insecticide that has been used against it in India (Saxena et al. 1989; Chawla and Joia 1992, Raju 1996; Sannaveerappanavar and Viraktamath 1997). Safe, efficient and eco-friendly control strategies can help reduce pesticide misuse in managing DBM and other brassica pests.

δ-endotoxins synthesized by the soil bacterium *Bacillus thuringiensis* (Bt) are highly specific to insect pests, and are being used as biopesticides to control several pest species (Lambert and Peferoen 1992; Schnepf et al. 1998) including DBM (Talekar and Shelton 1993). However, DBM had developed resistance to Bt formulations in the field in Central and North America, East and Southeast Asia and India (Diaz-Gomez et al. 2000; Ferre et al. 1991; Hama 1992; Mohan and Gujar 2003; Perez and Shelton 1997; Shelton et al. 1993; Syed 1992; Tabashnik et al. 1990; Zhao et al. 1993). However, the DBM populations that had developed resistance to *B. t.* subsp. *kurstaki* were found to be susceptible to *B. t.* subsp. *aizawai* (Talekar and Shelton 1993, Syed 1992), because *B. t.* subsp. *aizawai* has additional toxins such as Cry1C. Hence, it has been proven that the same population reacts differently to various Bt toxins. Recently, susceptibility of various field collected populations of *P. xylostella*, *C. binotalis*, *Hellula undalis*, *Pieris brassicae*, *P. rapae*, and *Diacrisia obliqua* in Australia, China, India, Indonesia, Taiwan and USA to Cry1Ba2 and Cry1Ca4 were determined (Shelton et al. 2009). In India, Cry1Ba2 was screened against 18 populations of DBM whereas Cry1Ca4 was screened

against 13 populations from different parts of the country. However, this study included only one population of *C. binotalis* from Delhi (North India) for both the toxins.

As there is no comprehensive information available on the baseline susceptibility of DBM to different Bt toxins in West Bengal, which is the largest cabbage and vegetable producer in India, we intended to do it in West Bengal. In addition, the baseline susceptibility of CHC to different Bt toxins is not available in South India, therefore Karnataka, where CHC is one of the serious pests on vegetable brassicas, was chosen. Gujarat, another intensive vegetable brassica producing state, also was selected. Thus, three Bt δ-endotoxins (Cry1Ac, Cry1Ba2 and Cry1Ca4) against DBM from Gujarat, Karnataka and West Bengal, and against CHC in Karnataka were screened. This is a timely study; attempts are underway to develop and introduce insect resistant transgenic vegetable brassicas with pyramided *cry* genes in India (Russell et al. 2008).

MATERIALS AND METHODS

DBM colonies were established at the Entomology laboratory of University of Agricultural Sciences, Bangalore from insects collected from fields in Gujarat (Anand), Karnataka (Bangalore) and West Bengal (Bolpur), that had not been sprayed with Bt formulations. The larvae had no contact with Bt toxins in rearing facilities and they were reared on potted mustard plants at 27±1°C, and 70±10% RH, L14:D10 until pupation. On pupation, they were placed inside cages. Upon emergence, adults were maintained on a 10% (wt:vol) honey solution dispensed on cotton wool placed inside the cages for mating and egg laying. Collected eggs were disinfected with 10% formalin. When the eggs hatched, the neonate larvae were transferred to new cages with potted mustard plants for multiplication. CHC colonies were also established from field-collected insects in Bangalore and they were reared similar to DBM. The early second instar larvae were used for bioassays.

Three lepidopteran-specific Bt δ-endotoxins (Cry1Ac, Cry1Ba2 and Cry1Ca4) were used in the bioassay. Five widely spaced concentrations for each toxin were used in the preliminary range finding tests. Six to seven concentrations of each δ-endotoxin, which could cause 10-90% mortality according to the preliminary range finding tests, plus an untreated control were included in each bioassay. Insect bioassays were performed using leaf dip bioassay IRAC method no. 7. Whole mustard leaves were cut into a series of 3 cm disks and dipped individually in the test concentration of a particular Bt toxin for 5 s with gentle agitation. For the untreated control, distilled water was used for dipping. An additional wetter was used at 0.1% for Bt toxin solutions as well as for the untreated control for better adherence to the surface of the mustard leaf disks. The disks were removed and placed on a mesh surface to dry for 2 h. Once the disks had dried, they were placed in plastic cups at the rate of two disks per cup. Five recently moulted, second instar larvae were added to each cup, so that a minimum of 50 larvae were used per dose, divided between at least 10 cups. The cups were stored in an area where they were not exposed to direct sunlight at a mean temperature of 27±1°C. A first assessment was made at 72 h, when the leaves were changed for fresh leaves treated with the appropriate Bt toxin. The cups were held for a further period before the final assessment, either for 72 h or until larvae in the untreated control had moulted again. The whole experiment was replicated at least three times. The results were expressed as percentage mortalities, correcting for untreated control mortalities using Abbott's formula (Abbott 1925). Assays that recorded more than 10% mortalities in untreated control were eliminated. The lethal concentrations causing 50% mortality (LC_{50}), 90% mortality (LC_{90}), their 95% fiducial limits (FL) and the slope value of probit line were assessed according to the probit analysis methodology using the statistical program LdP line (Ehab Mostofa Bakr, Cairo, Egypt).

RESULTS AND DISCUSSION

Cry1Ac was toxic to larvae of *P. xylostella* with LC_{50} (ppm) values ranging from 0.05 (Anand F_3, Bangalore F_3 and Bolpur $F_1$and F_3) to 0.17 (Anand F_1) among the three field populations tested (Table 1). There were significant differences in susceptibility between some populations, based on non-overlapping 95% FL values of the LC_{50}. Of the tested populations, Anand F_1 was threefold more tolerant than Anand F_3, Bangalore F_3 and Bolpur F_1 and F_3 populations. There was no significant difference in the LC_{50} values among the F_2 and F_3 generations of Anand population. The LC_{50} values among the F_1, F_2 and F_3 generations of the Bangalore population were significantly different. However, there was no significant difference in the LC_{50} values among the F_1, F_2 and F_3 generations of the Bolpur population. There was no earlier report available on the susceptibility of *P. xylostella* from these locations. However, susceptibility results from Andhra Pradesh, Delhi, Haryana, Jharkhand, Punjab and Tamil Nadu had shown that the LC_{50} values ranged between 0.01 and 0.32 ppm (Phani Kumar and Gujar 2005). Studies from Karnataka had shown that *P. xylostella* populations from Belgaum, Bidar, Dharwad and Haveri were highly susceptible to Biobit®, a formulation containing Cry1Aa, Cry1Ab, Cry1Ac, Cry2A and Cry2B toxins (Vastrad et al. 2004). Since *Bacillus thuringiensis* formulations have not yet been widely used in these regions, *P. xylostella* is still susceptible to Cry1Ac.

Cry1Ba2 was toxic to larvae of *P. xylostella* with LC_{50} (ppm) values ranging from 0.05 (Bangalore F_3) to 0.16 (Bangalore F_1) among the three field populations tested (Table 2). Bangalore F_1 was three fold more tolerant whereas Anand F_2 and Bolpur F_1 were two fold tolerant than Bangalore F_3 population. There were no significant differences in the LC_{50} values among the F_1, F_2 and F_3 generations of both Anand and Bangalore populations,

Table 1. Toxicity of Cry1Ac to field collected strains of *P. xylostella* larvae in leaf-dip assay at 72-h reading

Insect strain	State of collection	Gen (F)	Slope (SE)	LC_{50} (95% FL) ppm	LC_{90} (95% FL) ppm	X^2 (df)
Anand	Gujarat	F_1	0.76 (0.05)	0.17 (0.12 – 0.23)	8.15 (4.77 – 15.85)	5.25 (5)
		F_2	0.82 (0.05)	0.09 (0.07 – 0.13)	3.40 (2.10 – 6.09)	12.31 (5)
		F_3	0.73 (0.05)	0.05 (0.02 – 0.18)	3.14 (1.74 – 33.32)	29.32 (5)
Bangalore	Karnataka	F_1	0.74 (0.04)	0.09 (0.05 – 0.17)	4.86 (2.50 – 14.70)	15.06 (5)
		F_2	0.93 (0.05)	0.14 (0.07 – 0.30)	3.40 (1.86 – 11.23)	24.57 (5)
		F_3	1.04 (0.06)	0.05 (0.04 – 0.06)	0.85 (0.57 – 1.38)	8.54 (5)
Bolpur	West Bengal	F_1	0.71 (0.05)	0.05 (0.04 – 0.07)	3.20 (1.86 – 6.24)	5.27 (5)
		F_2	0.80 (0.05)	0.07 (0.04 – 0.13)	2.70 (1.43 – 7.75)	15.04 (5)
		F_3	0.83 (0.06)	0.05 (0.04 – 0.07)	1.70 (1.02 – 3.23)	6.94 (5)

Table 2. Toxicity of Cry1Ba2 to field collected strains of *P. xylostella* larvae in leaf-dip assay at 72-h reading

Insect strain	State of collection	Gen (F)	Slope (SE)	LC_{50} (95% FL) ppm	LC_{90} (95% FL) ppm	X^2 (df)
Anand	Gujarat	F_1	0.88 (0.06)	0.09 (0.05 – 0.18)	2.64 (1.42 – 7.94)	14.05 (5)
		F_2	1.00 (0.06)	0.11 (0.06 – 0.18)	2.07 (1.20 – 4.81)	13.09 (5)
		F_3	0.81 (0.05)	0.08 (0.04 – 0.18)	3.11 (1.63 – 10.86)	23.89 (5)
Bangalore	Karnataka	F_1	0.72 (0.05)	0.16 (0.05 – 0.51)	9.45 (5.15 – 91.82)	28.81 (5)
		F_2	0.92 (0.06)	0.06 (0.03 – 0.12)	1.59 (0.87 – 4.69)	15.31 (5)
		F_3	0.89 (0.05)	0.05 (0.03 – 0.09)	1.52 (0.87 – 3.49)	12.81 (5)
Bolpur	West Bengal	F_1	0.85 (0.06)	0.12 (0.08 – 0.16)	3.76 (2.30 – 6.94)	8.49 (5)
		F_2	0.91 (0.06)	0.06 (0.03 – 0.15)	1.53 (0.83 – 7.22)	24.41 (5)
		F_3	0.78 (0.05)	0.06 (0.02 – 0.13)	2.43 (1.24 – 11.84)	18.77 (5)

Table 3. Toxicity of Cry1Ca4 to field collected strains of *P. xylostella* larvae in leaf-dip assay at 72-h reading

Insect strain	State of collection	Gen (F)	Slope (SE)	LC_{50} (95% FL) ppm	LC_{90} (95% FL) ppm	X^2 (df)
Anand	Gujarat	F_1	0.61 (0.04)	0.05 (0.02 – 0.10)	5.91 (2.72 – 25.77)	16.95 (6)
		F_2	0.86 (0.05)	0.14 (0.07 – 0.31)	4.33 (2.32 – 14.97)	24.97 (6)
		F_3	0.78 (0.05)	0.06 (0.04 – 0.08)	2.70 (1.64 – 4.93)	11.70 (6)
Bangalore	Karnataka	F_1	0.73 (0.04)	0.10 (0.07 – 0.14)	5.84 (3.45 – 11.05)	11.07 (6)

		F_2	0.70 (0.04)	0.07 (0.03 – 0.19)	4.91 (2.46 – 27.18)	30.23 (6)
		F_3	0.75 (0.04)	0.11 (0.04 – 0.34)	5.47 (2.90 – 40.06)	42.04 (6)
Bolpur	West Bengal	F_1	0.61 (0.04)	0.06 (0.02 – 0.14)	7.51 (3.48 – 41.38)	21.88 (6)
		F_2	1.01 (0.07)	0.09 (0.04 – 0.18)	1.58 (0.87 – 5.74)	12.21 (4)
		F_3	0.91 (0.05)	0.04 (0.03 – 0.05)	0.98 (0.67 – 1.51)	6.57 (6)

Table 4. Toxicity of Cry1Ac, Cry1Ba2 and Cry1Ca4 to field collected *C. binotalis* larvae from Bangalore, Karnataka in leaf-dip assay at 72-h reading

Toxin	Gen (F)	Slope (SE)	LC_{50} (95% FL) ppm	LC_{90} (95% FL) ppm	X^2 (df)
Cry 1Ac	F_1	0.67 (0.05)	0.01 (0.008 – 0.018)	1.00 (0.57 – 2.02)	7.22 (5)
	F_2	0.74 (0.05)	0.02 (0.01 – 0.03)	1.01 (0.61 – 1.87)	6.86 (5)
	F_3	0.74 (0.06)	0.004 (0.003 – 0.006)	0.23 (0.13 – 0.47)	7.36 (4)
Cry 1B	F_1	0.99 (0.06)	0.07 (0.03 – 0.14)	1.37 (0.77 – 4.48)	18.41 (5)
	F_2	0.98 (0.06)	0.03 (0.02 – 0.07)	0.67 (0.38 – 2.24)	18.29 (5)
	F_3	0.95 (0.06)	0.04 (0.02 – 0.07)	0.84 (0.46 – 2.49)	14.66 (5)
Cry 1C	F_2	0.88 (0.05)	0.04 (0.03 – 0.05)	1.10 (0.71 – 1.86)	11.86 (6)
	F_3	0.70 (0.04)	0.03 (0.01 – 0.08)	2.13 (1.05 – 11.85)	33.81 (6)

although LC_{50} values among the F_1, F_2 and F_3 generations of Bolpur population were significantly different. Our findings were consistent with an earlier report. *Plutella xylostella* Bangalore populations recorded a LC_{50} value of 0.01-0.21 ppm, and a Vadodara (Gujarat) population recorded a LC_{50} value of 0.05 ppm (Shelton et al. 2009). The same study stated that most of the Indian populations from different geographical locations such as Andhra Pradesh, Assam, Delhi, Haryana, Himachal Pradesh, Jharkhand, Maharashtra, Uttarakhand and Uttar Pradesh recorded a LC_{50} value of 0.01-0.46 ppm. Since no *B. thuringiensis* formulation containing Cry1Ba2 is available for use in India, *P. xylostella* is highly susceptible to this toxin.

Cry1Ca4 was equally toxic to larvae of *P. xylostella* with LC_{50} (ppm) values ranging from 0.04 (Bolpur F_3) to 0.14 (Anand F_2) among the three field populations tested (Table 3). The LC_{50} values among the F_1 and F_3 generations of Anand population were significantly different from the F_2 generation. Similar results were obtained for the Bolpur population. There were no significant differences in the LC_{50} values among the F_1, and F_2 generations of the Bangalore population, although the LC_{50} value for the F_3 generation was significantly different. Our findings were comparable with an earlier report. *Plutella xylostella* Bangalore populations, as well as most of the other populations from different geographical locations in India such as Andhra Pradesh, Assam, Delhi, Haryana, Himachal Pradesh, Jharkhand, Maharashtra, Uttarakhand and Uttar Pradesh, recorded a LC_{50} value of 0.01-0.43 ppm (Shelton et al. 2009). Since no *B. thuringiensis* formulation containing Cry1Ca4 is available for use in India, *P. xylostella* is still highly susceptible to this toxin.

Cry1Ac was more toxic to larvae of *C. binotalis* (Bangalore population) with LC_{50} (ppm) values ranging between 0.004 and 0.02 than Cry1Ba2 and Cry1Ca4, which recorded LC_{50} values ranging from 0.03 to 0.07 ppm (Table 4). Although the susceptibility of *C. binotalis* to Cry1Ac is unavailable, it was highly susceptible to *B. t.* subsp. *kurstaki* formulations having Cry1A as major toxins in India (Malathi and Sriramulu 2000). An earlier study documented that *C. binotalis* (Delhi population) recorded a LC_{50} value of 0.07 ppm to Cry1Ba2 (Shelton et al. 2009), which is similar to our findings. However, the same study has indicated that the susceptibility of *C. binotalis* (Delhi population) to Cry1Ca4 was almost 45-60 fold lower (1.89 ppm) than our LC_{50} values (0.03-0.04 ppm).

CONCLUSION

All the field populations of *P. xylostella* (Anand, Bangalore and Bolpur) and *C. binotalis* (Bangalore) were highly susceptible to the tested *B. thuringiensis* toxins *viz.,* Cry1Ac, Cry1Ba2 and Cry1Ca4.

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Toxicity and biological effects of neem limonoids on diamondback moth, *Plutella xylostella*

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ABSTRACT

Azadirachtin, salannin, deacetylgedunin, gedunin, 17-hydroxyazadiradione, and deacetylnimbin are the groups of neem limonoids from the neem tree (*Azadirachta indica* A. Juss) that have attracted considerable attention for the biological activities on the vast number of insects. In the present study, the biological and toxicity effects of aforementioned limonoids were tested against diamondback moth, *Plutella xylostella* (L.). Considerable mortality was evident after the treatment of 3rd instar larvae of *P. xylostella*, and among the neem limonoids, azadirachtin caused higher mortality than other neem limonoids. Median lethal concentrations (LC_{50} and LC_{90}) were determined for the 3rd instar larvae. Antifeedancy was also higher with azadirachtin than other compounds. Prolonged larval and pupal durations and concomitant decrease in adult durations were also noted. Prolongation of the developmental period of *P. xylostella* after the treatment is mainly attributed to the interference of neem limonoids with the endocrine system. Significant reduction in fecundity, egg hatchability and adult longevity were mainly due to the effect of neem limonoids on reproduction, especially on the maturation of oocytes and increased mortality of eggs. These results are discussed relative to the structure of activity relationships of neem limonoids (especially azadirachtin) and their bioactivities against insects

Keywords

neem limonoids, *Plutella xylostella,* toxicity, antifeedancy, development

INTRODUCTION

The diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae) is a major pest of cabbages and other cruciferous crops throughout the world (Talekar and Shelton 1993), and it has been reported in at least 128 countries or territories (Salinas 1972). The global importance of *P. xylostella* is reflected in its control cost of approximately 1 billion US$ annually (Talekar and Shelton 1993; Verkerk and Wright, 1996; Sarfraz et al. 2007). Insecticide resistance and control failures for *P. xylostella* are now common in many parts of the world (Talekar and Shelton 1993; Shelton et al. 2000; Zhao et al. 2006). To control this insecticide-resistant insects, many researchers are attempting to develop effective alternative control methods, including resistant cultivars (Dickson et al.1990), cultural practices such as sprinkler irrigation (Talekar et al.1986), synthetic sex pheromone (Nemoto et al. 1992), microbial agents (George and Thomas 1992), selective insecticides having new mode-of-action (Schmutterer 1992), and natural enemies (Lim 1986; Talekar et al.1992).

The Indian neem tree, *Azadirachta indica* A. Juss (Meliaceae) is a promising source of botanical insecticides. Due to their relative selectivity, neem products can be recommended for many integrated pest management (IPM) programs (Schmutterer 1990; Ishida et al. 1992; Koul and Wahab 2004; Senthil Nathan et al. 2004; 2005 a,b). About 150 such compounds have now been described (Akhila and Rani 1999), most of them found in very small quantities in various parts of the tree. Only about one-third of them have been tested for biological activity and none has shown greater activity than the azadirachtin group.

It is generally believed that the bioactivity of neem is due to the azadirachtin (and other complex limonoids) content (Butterworth and Morgan 1968). Limonoids are tetranortriterpenes and secondary metabolites produced in plants of the order Rutales. Limonoids are most often found in the family Meliaceae and less frequently in the families Rutaceae and Cncoraceae, within this order (Champagne et al. 1989; 1992). Limonoids are described as modified triterpenes, having a 4, 4, 8 trimethyl-17 furgnyl steroid skeleton (Fig.1). Arrangements of sub-groups and ring structures within this basic building block provide a host of characteristics that have generated interest in this plant product (Connolly 1983). In the past, approaches to the qualitative and quantitative analysis of azadirachtin and other neem triterpenoids form different sources mostly included reversed-phase high-performance liquid chromatography (RP-HPLC), because of the polarity of the neem compound (Sundaram and Curry 1993; Schaaf et al. 2000).

Although there are several potential insecticidal compounds in neem extracts, the principle active ingredient is the tetranortriterpenoid (azadirachtin), which has a profound effect on the feeding, growth, molting and reproduction of insects (Lee et al. 1991; Murugan et al. 1998), though azadirachtin also inhibits hormones (Malezewaka et al. 1988) and enzyme activity (Babu et al. 1996; Murugan et al. 1996).The present investigation was undertaken to study the effect of neem limonoids on toxicity, growth and reproduction of *P.xylostella*

MATERIALS AND METHODS

The diamondback moth (DBM), *Plutella xylostella* larvae were collected from cabbage fields in Thondamuthur, Coimbatore District (11°0′45″N

76°58′17″E), Tamil Nadu, India and mass-cultured in laboratory. The larvae were fed with cabbage leaves *ad libitum* at 28±2ºC, 16:8 L:D, 75% RH.

Neem limonoids

Six neem limonoids (Fig.1) (purity > 99%) namely azadirachtin, salannin, deacetylgedunin, gedunin, 17-hydroxyazadiradione and deacetylnimbin were obtained from M. Ishida, Central Research Laboratories, Taiyo Kagaku Co. Ltd., Japan. They were dissolved in isopropanol and different concentrations were prepared by dilution with isopropanol.

Biological parameters

Post-embryonic development was assessed by separating freshly laid eggs. Early first instar larvae were treated for 3-d with the individual and combined treatment at neem limonoids and observations were made on the duration of total larval stages, pupal period and adult longevity. The newly emerged adult male and female insects (from treated larvae) were separated and fed on 10% sucrose solution fortified with a few drops of vitamin mixture (MULTIDEC DROPS obtain from Ashoka Pharmaceutical, Chennai-600 010, Tamil Nadu, India) to enhance oviposition. After 2-d, these treated adult males (10-20 individuals in each experiment) were maintained with the same number of treated females of the same age. Mating was observed and fecundity as well as egg hatchability were measured.

Antifeedant bioassay

Feeding deterrence index was estimated by using a leaf choice test (Isman et al. 1990; Khan et al. 1996). In a 15 cm diameter petridish lined with a moist filter paper disc, 5 cm long strips of cut-leaf from cabbage plants were treated with 5 ml of aqueous solutions of the neem limonoids emulsified with Triton-X100 (0.1%). Controls were treated with isopropanol alone. The treated cut-leaves were dried at room temperature and then 6 h starved third instar *P. xylostella* larvae were introduced into each arena containing one treated and untreated leaf discs in alternate position lined with moist filter paper disc. Experiments were carried out with 10 larvae per concentration. Each experiment was replicated five times. Consumption was recorded using a digital leaf area meter (Model LI-3000, Li-cor, USA) after 12 h. The index of feeding deterrency was calculated using the formula (C-T)/(C+T)X100, where C is the consumption of control cut-leaf and T is the treated cut-leaf.

Toxicity bioassay

Various concentrations of neem limonoids were applied evenly to cabbage leaves with a fine brush. The treated leaves were air-dried. Newly molted 3rdinstar larvae of *P. xylostella* obtained from the laboratory colony were starved for 6 h and transferred to plastic containers (6.5 cm height X 5.5 cm diameter) containing treated or controlled cabbage leaves (two larvae/container and 30 larvae/concentration) and allowed to feed for 48 h. The mortality was recorded at every 12 h and final mortality was recorded after 48 h and corrected for control mortality using Abbott's formula (Abbott 1925). The lethal concentrations causing 50% and 90% mortalities (Lc_{50} and Lc_{90}) were calculatedusing probit analysis (Finney 1971).

Data analysis

LC_{50} and LC_{90} values and the 95% fiducial limits were calculated from a log dosage-probit mortality computer software program (SPSS 9.0 Windows data editor). The mean differences among the treatments were compared using analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) (Alder and Rossler 1997).

RESULTS AND DISCUSSION

A. indica, commonly known as neem possesses a vast array of bioactive phytochemicals that exhibit potent insecticidal properties. All the limonoids showed a positive dose dependent antifeedant activity. Of six limonoids tested against *P. xylostella*, azadirachtin showed the highest antifeedent activity (Table 1). Azadirachtin at 1.00 ppm produced the highest feeding deterrence (89.4±7.7% in third instar larvae than all other limonoids. The reduced consumption of limonoid-treated leaves is likely to be the main cause of growth inhibition (Murugan et al. 1998). Inhibition of feeding behavior results from stimulation of deterrent receptors by azadirachtin often coupled with an inhibition of sugar receptors (Simmonds and Blaney 1984). In Lepidoptera, the antifeedant response is also correlated with increased neural activity of the chemoreceptors. In this study, the potent antifeedant activity was caused by azadirachtin, salannin, deacetylgedunin, gedunin, 17-hydroxyazdirdione and all these five compounds have some common structural feature such as a furan ring and an α, β–unsaturated ketone in their A–ring. Azadirachtin is by far the most potent antifeedant among the compounds, being more than ten times as effective as the second most potent antifeedant salannin. Azadirachtin is a highly oxidized tetranortriterpenoid with trans–connected A and B rings with an epoxide ring at position 13, 14 and a trilogl side chain at position 1. Three hydroxyl groups at position 7, 11, 20 are free in the azadirachtin molecule (Rembold 1989). These hydroxyl groups seem to be a main factor in determining the antifeedant activity (Ishida et al. 1992).

Table 2 shows the mortality of 3rd instar larvae after the treatment of neem limonoids and the lethal concentration (LC_{50} and LC_{90}) values of azadirachtin treatment at 0.25, 0.50 and 1.00 ppm concentrations were 0.240 ppm and 0.554 ppm, respectively. After the treatment of salannin at 0.25, 0.50 and 1.00 ppm concentration, the LC_{50} and LC_{90} values were 0.362 ppm and 1.145 ppm, respectively, whereas in other limonoid treatments, the lethal concentration values increased. Azadirachtin caused higher mortality than other limonoids and the toxicity at the highest concentration may be due to the toxic effects on the larval midgut epithelium, causing a disruption in membrane integrity and ultimately leading

to death of larvae (Nasiruddin and Mordue (Luntz) 1993).

Tables 3 and 4 showed the biological parameters of *P. xylostella* after the treatment of neem limonoids. Neem limonoids greatly affected the larval, pupal and adult stages, and prolongation of larval and pupal durations were evident. The total larval duration at 1.00 ppm of azadirachtin, salannin, deacetylgedunin, gedunin, 17-hydroxyazadiradione and deacetylnimbin were 27.6±0.13, 24.6±0.08, 22.5±0.84, 20.4±0.1, 19.6±0.83 and 19.1±0.08 days, respectively. Similarly pupal duration was also significantly extended. After the treatment of neem limonoids, the longevity of male and female adults was reduced. The fecundity was also decreased after the treatment of neem limonoids. In the present study, neem limonoids greatly affected the total number of eggs laid by *P. xylostella*. Koul (1984) observed the trophocyte damage after administration of azadirachtin to *Dysdercus koenigii* (F.) female. Likewise, azadirachtin affected ovaries in the nymphs of *Oncopeltus fasciatus* (Dorn et al. 1986) and in locusts (Rembold et al. 1987). A similar finding was noted in adult *Locusa migratoria* after azadirachtin treatment (Rembold 1984; Rembold et al. 1987). In the present study, azadirachtin caused significant mortality to all the larval instars of *P. xylostella* tested and the mortality was found to be dose dependent. Larval pupal intermediates were closely linked with poor feeding and concomitant lack of growth. Mordue et al. (1986) showed that the azadirachtin resulted in a lack of growth and an apparent blockage of ecdysteroid release.

In the present study, after the treatment with neem limonoids, the larval and pupal durations were extended. The growth inhibition may be due to the hormonal interference on the reproductive process of insects. Earlier studies made on different insects such as *O. fasciatus, Epilachna varivestis,* and *L. migratoria* showed complete molt inhibition due to a blockage of ecdysteroid synthesis and release, a delay in appearance of the ecdysteroid peak, and a slow subnormal decline in this peak (Redfern et al. 1982; Sieber and Rembold 1983; Schlüter et al. 1985; Mordue (Luntz) et al. 1986). In the present, study the reduced fecundity and egg hatchability may be due to the interference of the uptake of fat body protein by the ovary. The adverse effects of azadirachtin on ovarian and testes development, fecundity and fertility are an important component of overall toxicity and insect control (Karnavar 1987; Mordue (Luntz) and Blackwell 1993; Mordue (Luntz) 2000).

CONCLUSION

Neem limonoids exhibited antifeedent, growth inhibition and affected the reproductive performance of *P. xylostella*. Hence, neem molecules could perhaps be useful for IPM programs. Moreover, insects may not develop resistance against neem, and is also safer to the natural enemy complex. Earlier studies demonstrating resistance and desensitization suggest that these problems can be avoided by using the mixture of neem triterpenoids. Feng and Isman (1995) showed that the peach potato aphid, *Myzus persicae* developed resistance to pure azadirachtin over 40 generations, but the same did not happen with neem seed extract. In another study with *S. litura* larvae on cabbage, the larvae became desensitized to pure azadirachtin in both choice and non-choice tests, but did not desensitize to the seed extract even though the latter contained the same amount of azadirachtin (Bomford and Isman 1996; Murugan and Vanithakumari 2009).

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Table 1. Antifeedant activity of neem limonoids against third instar larvae of *Plutella xylostella*

Neem Limonoids	Concentration (ppm)	Feeding deterrence (%)
Control		0.05±0.02[a]
Azadirachtin	0.25	51.2±1.1[c]
	0.50	63.0±0.9[b]
	1.00	89.2±0.6[b]
Salannin	0.25	46.8±0.3[a]
	0.50	55.6±0.6[b]
	1.00	76.4±0.5[a]
Deacetylgedunin	0.25	28.0±0.3[a]
	0.50	37.4±0.5[a]
	1.00	58.0±0.3[a]
Gedunin	0.25	13.0±0.2[a]
	0.50	23.8±0.5[a]
	1.00	38.2±0.7[b]
17-Hydroxyazadiradione	0.25	11.7±0.9[a]
	0.50	21.3±1.0[a]
	1.00	32.0±1.07[c]
Deacetylnimbin	0.25	8.4±0.1[a]
	0.50	14.0±1.4[d]
	1.00	20.6± 0 .7[b]

± within the table shows the (SEM); within a column means followed by the same letter (s) are not significantly different at 5% level by DM

Table 2. Larvicidal and pupicidal effect of neem limonoids against third instar larvae of *Plutella xylostella*

Neem Limonoids	% of larval and pupal mortality			LC_{50}	LC_{90}	Regression equation	95% Fiducial limit				Chi square value
	Concentration of neem limonoid (ppm)						LCL		UCL		
	0.25	0.5	1.0				LC_{50}	LC_{90}	LC_{50}	LC_{90}	
Azadirachtion	52	85[hi]	100[j]	0.240	0.554	Y=-0.980 +4.079X	0.159	0.489	0.292	0.671	0.127
Salannin	40[f]	63[e]	84[d]	0.362	1.145	Y=-0.593 +1.638X	0.229	0.977	0.458	1.446	1.097
Deacetylgedunin	36[de]	58[cd]	81[cd]	0.432	1.224	Y=-0.700 +1.618X	0.315	1.044	0.525	1.549	0.857
Gedunin	23[b]	45[b]	71b[c]	0.642	1.414	Y=-1.066 +1.661X	0.552	1.210	0.743	1.774	1.267
17-Hydrocyazadiradione	19[a]	40[a]	65[a]	0.734	1.533	Y=-1.177 +1.604X	0.640	1.302	0.855	1.952	1.525
Deacetylnimbin	21[a]	43[a]	67[a]	0.693	1.503	Y=-1.097 +1.582X	0.599	1.275	0.808	1.919	1.732

Within a column means followed by the same letter (s) are not significantly different at 5% level by DM

Table 3. Effect of Neem limonoids on the larval development of *Plutella xylostella*

Neem Limonoids	Larval Duration (days)				Total larval Duration (days)
	I	II	III	IV	
Control	4.2±0.48	4.0±0.35	4.3±0.27	4.7±0.30	17.6±0.11
Azadirachtion					
0.25	4.9±0.30^{a}	4.8±0.34^{b}	5.2±0.48^{d}	5.9±0.41^{a}	19.9±0.15^{b}
0.50	5.6±0.35^{b}	5.3±0.46^{e}	5.8±0.37^{a}	6.3±0.50^{c}	22.6±0.08^{a}
1.00	6.6±0.35^{b}	6.7±0.28^{a}	6.3±0.43^{b}	7.4±0.76^{h}	27.6±0.13^{b}
Salannin					
0.25	4.7±0.30^{a}	4.5±0.35^{a}	4.9±0.34^{a}	5.6±0.49^{c}	19.7±0.11^{b}
0.50	5.3±0.38^{c}	5.0±0.48^{e}	5.3±0.37^{b}	6.0±0.47^{c}	22.0±0.11^{b}
1.00	5.8±0.33^{a}	6.0±0.47^{e}	6.3±0.43^{c}	6.5±0.38^{a}	24.6±0.08^{a}
Deacetylgedunin					
0.25	4.5±0.39^{a}	4.3±0.25^{a}	4.7±0.30^{a}	5.3±0.51^{e}	18.7±0.15^{c}
0.50	5.0±0.48^{c}	4.8±0.30^{b}	4.9±0.34^{a}	5.4±0.38^{b}	20.1±0.1^{a}
1.00	5.4±0.38^{a}	5.5±0.37^{d}	5.6±0.50^{e}	5.9±0.33^{a}	22.5±0.84^{e}
Gedunin					
0.25	4.4±0.35^{c}	4.3±0.25^{a}	4.5±0.37^{b}	5.1±0.48^{c}	18.4±0.08^{a}
0.50	4.8±0.27^{a}	4.5±0.48^{f}	4.7±0.30^{a}	5.2±0.46^{c}	19.2±0.08^{a}
1.00	5.0±0.37^{c}	4.9±0.34^{c}	5.0±0.62^{h}	5.5±0.38^{a}	20.4±0.1^{c}
17-Hydroxyazadiradione					
0.25	4.3±0.25^{a}	4.2±0.49^{f}	4.4±0.35^{a}	5.0±0.59^{d}	17.9±0.11^{a}
0.50	4.6±0.37^{c}	4.3±0.25^{a}	4.6±0.37^{b}	5.1±0.48^{a}	18.6±0.11^{a}
1.00	4.8±0.27^{a}	4.6±0.37^{c}	4.9±0.34^{a}	5.3±0.51^{b}	19.6±0.83^{e}
Deacetylnimbin					
0.25	4.3±0.25^{a}	4.0±0.54^{g}	4.4±0.35^{b}	4.8±0.27^{a}	17.5±0.84^{e}
0.50	4.5±0.48^{f}	4.3±0.25^{a}	4.5±0.48^{e}	5.0±0.37^{c}	18.3±0.11^{b}
1.00	4.7±0.30^{b}	4.5±0.48^{f}	4.7±0.30^{a}	5.2±0.45^{d}	19.1±0.08^{a}

±SD within a column means followed by the same letter (s) are not significantly different at 5% level by DMRT

Table 4. Effect of neem limonoids on the pupal period, adult longevity, fecundity and egg hatchability of *Plutella xylostella*

Neem Limonoids	Pupal Period (days)	Adult Longevity (days)		Fecundity (No. of Eggs)	Egg Hatchability (%)
		Male	Female		
Control	5.8±0.80	12.0±1.14	16.0±0.16	158±1.87	96.8
Azadirachtin					
0.25	7.0±0.08[a]	10.3±0.11[a]	12.1±0.16[d]	102±1.22[e]	85.2
0.50	8.4±0.08[a]	9.1±0.11[a]	11.3±0.08[a]	83±1[a]	72.2
1.00	9.7±0.15[c]	7.0±0.13[a]	10.1±0.07[a]	68±1[a]	47.0
Salannin					
0.25	6.8±0.11[c]	10.8±0.1[e]	13.9±0.19[b]	110±1.22[e]	88.1
0.50	8.0±0.12[c]	10.2±0.08[a]	12.6±0.1[f]	88.8±1.3[a]	77.5
1.00	9.5±0.1[a]	9.1±0.11[b]	11.1±0.15[a]	71.8±1.3[a]	58.3
Deacetylgedunin					
0.25	6.5±0.08[a]	11.3±0.08[a]	14.6±0.08[a]	118±1.22[g]	91.5
0.50	7.8±0.11[b]	10.9±0.11[b]	13.2±0.11[b]	105.8±0.83[a]	84.9
1.00	9.0±0.11[b]	10.0±0.07[a]	12.0±0.08[a]	80±1.22[g]	71.2
Gedunin					
0.25	6.3±0.11[b]	11.6±0.08[a]	15.0±0.08[a]	124±1[a]	93.5
0.50	7.5±0.08[a]	11.5±0.11[c]	14.0±0.11[b]	111±1[a]	88.2
1.00	8.6±0.11[b]	10.4±0.11[c]	13.1±0.08[a]	88±2.12[f]	78.4
17-Hydroxyazadiradione					
0.25	6.1±0.08[a]	11.8±0.1[e]	15.6±0.1[g]	134±1.22[e]	95.5
0.50	7.3±0.13[b]	11.0±0.08[a]	15.0±0.08[a]	121±1[a]	92.5
1.00	8.2±0.08[a]	10.6±0.08[a]	13.7±0.17[c]	99±1[a]	84.8
Deacetylnimbin					
0.25	5.9±0.16[c]	12.0±0.19[d]	16.0±0.16[b]	147.6±1.14[c]	95.2
0.50	6.6±0.11[a]	11.4±0.11[b]	15.4±0.1[g]	130±1.14[c]	91.5
1.00	7.8±0.11[a]	11.0±0.08[a]	14.0±0.11[a]	108±1[a]	85.1

±SD within a column means followed by the same letter (s) are not significantly different at 5% level by DMRT

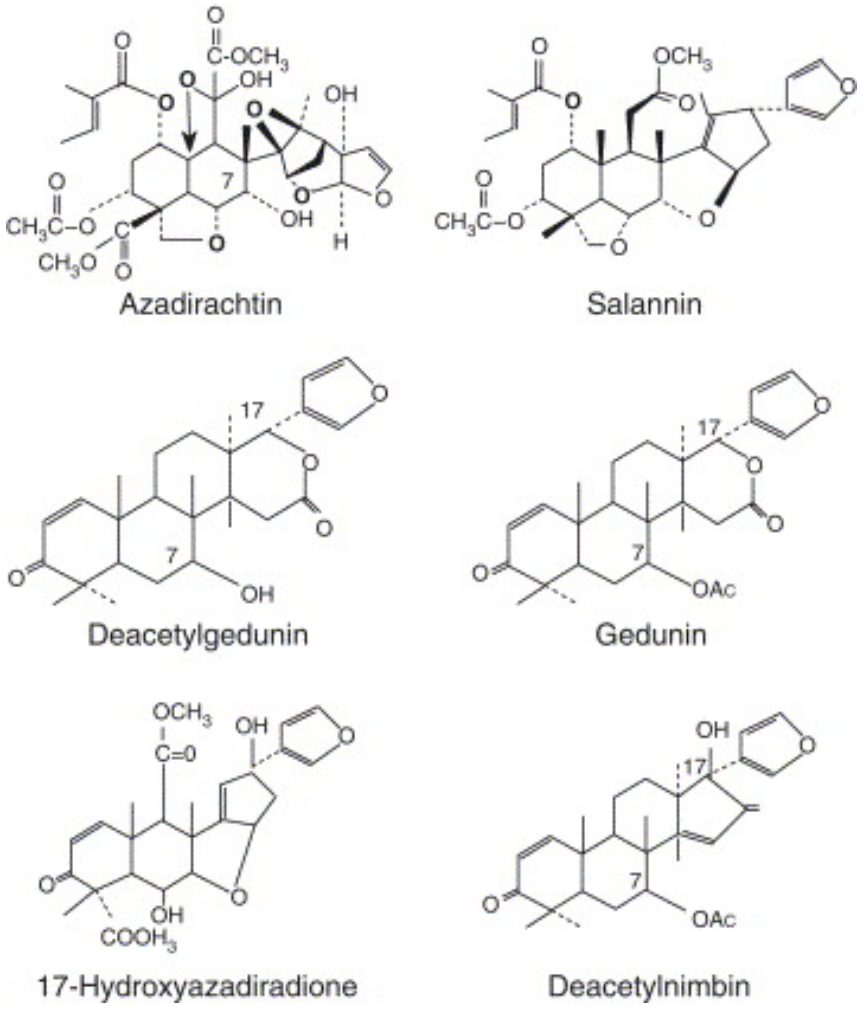

Figure 1. Structure of neem limonoids

Why is a crude extract of neem superior to commercial neem formulations? A field test against *Plutella xylostella* (L.) (Lepidoptera : Plutellidae) in cabbage

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ABSTRACT
A study was conducted to ascertain the efficacy of seven commercial neem formulations (CNFs) and aqueous neem seed kernel extract (NSKE: 4%) under laboratory and farmers' field conditions against diamondback moth (DBM), *Plutella xylostella* in vegetable cabbage. Bioassays were carried out using standard leaf dip method. In the field tests, indoxacarb (0.006 %) was used as the additional check. Treatments were imposed six times at approximately 10 -12 days interval starting from 12 days after transplanting. NSKE and indoxacarb treated plots maintained continuous low levels of DBM per plant throughout the cropping period, and were superior to all other treatments. The CNFs were generally on par with each other. NSKE registered the highest per cent (94.44%) harvestable heads. However, indoxacarb treated plots registered the highest (123.33±14.03 kg/plot) yield followed by NSKE (109.07±9.66 kg/plot). Thus CNFs were not up to the mark compared to NSKE and indoxacarb treated plots. Azadirachtin applied per unit spray volume in the form of various CNFs or NSKE was observed to be strongly correlated to the mean density of DBM per day per plant and the yield per plot. CNFs at the recommended doses accounted for lower azadirachtin content per unit of spray volume compared to NSKE and at the present recommended doses, all CNFs were twice as expensive as NSKE. Therefore, attempts at increasing the doses of azadirachtin in the spray solutions to match NSKE will only make them more expensive for field use. Thus, NSKE at 4 % is economical for the management of DBM in cabbage production system.

KEYWORDS
Plutella xylostella, Cabbage, Commercial Neem formulations, Azadirachtin, NSKE, Indoxacarb.

INTRODUCTION
Cabbage is an important vegetable cash crop for many farmers around cities in South India and it occupies four per cent of the area of all vegetables grown in the country. Due to introduction of heat tolerant varieties, it is now grown throughout the year. India is one among the leading producers of cabbage standing next only to China in the over all production of head cabbages. The crop is generally a 90 day crop after planting with a nursery period of around 20 to 25 days. Head formation is seen in about 35 to 40 days after planting and the heads can attain 5 kg or more in size (Chadha 2001).

Among the major hurdles for the production of healthy cabbage crop is the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). The larvae feed on all stages of the crop and feeding results in development of holes on the leaves, which enlarge as the plant grows. Larvae might attack and nibble at the growing primordia. Severe damage to primordia causes complete loss of the crop with an expected threshold levels of 4 to 7 larvae per plant (Srinivasan and Veeresh 1986). The larval incidence is of importance in greatly reducing the crop yield parameters such as number of harvestable heads, head diameter, head weight and ultimately the yield (Chelliah and Srinivasan 1986). DBM is of particular concern as a serious pest of crucifers since the insect is extremely difficult to manage. Its prolific breeding abilities, capacity to adopt to variable environmental conditions, short life cycle (Chelliah and Srinivasan 1986) and absence of effective natural enemies (Lim 1986) are believed to be the factors responsible for the insect to become a number one pest of cabbage farmers. In India, farmers completely depend on insecticides to manage DBM. Tropical countries where crucifers are grown throughout the year, DBM may complete up to 20 generations per year, and the sole reliance on insecticides for control facilitates the rapid build up of resistance (Talekar and Shelton 1993). To overcome the resistance, farmers often increase doses of insecticides, use mixtures of several chemicals and spray up to 25 times within a cropping season (Khan et al. 1991). These high levels of use of insecticides has caused DBM to become resistant to almost all insecticides in use and on an average, a new insecticide is expected to have a utility life of just two to three years in any cabbage production system.

Keeping these in mind, alternatives have been explored and home made neem seed kernel extract (NSKE) at the rate of 4% (W/V) has been recommended as the best solution to manage DBM in cabbage (Srinivasan and Krishnamoorthy 1993). Although, repeated tests have shown NSKE to be an excellent solution for the management of DBM under field conditions (Sannaveerappanavar and Viraktamath 1997; Shankaramurthy 2001), the recommendation is yet to

catch up with the farming community. Many reasons have been ascribed for the non-adoption of NSKE, and cumbersome procedure involved in the preparation of the extract is the major limiting factor (Pillai 2002).

Neem has been shown to be an excellent source of many insecticidal compounds and azadirachtin has been recognized as a potent chemical for insect control in many cropping systems (Schmutterer 1995; Kraus 1995). Consequently, many commercial neem formulations (CNFs) based on neem are currently available in the market in India, and can serve as potential replacements for NSKE (Ermel and Kleeberg 1995; Gahukar 1996). However, the concentration of the active ingredient, azadirachtin, varies greatly in these products ranging from 300 to 50,000 ppm and some of these products have no designated azadirachtin content. The present study was therefore planned to evaluate different commercial neem formulations of a range of concentrations available in the market for the management of DBM in cabbage.

Two approaches were used in the evaluation of the CNFs. First, a set of bioassays were carried out against DBM, to understand the relative insecticidal potential of the various CNFs and to compare their performance with that of the NSKE. Secondly, the same set of selected CNFs was evaluated under field conditions in a small plot trial along with NSKE and a chemical insecticide, indoxacarb as checks.

MATERIALS and METHODS

The test insecticides

Commercial neem formulations

A literature and internet survey revealed six grades of CNFs consisting of 300, 1500, 3000, 10000, 30000 and 50000 ppm of declared azadirachtin content to be available in the market. In addition, some powder formulations of neem of unspecified azadirachtin content were also available. One product each from these categories of the CNFs along with one neem seed powder formulation of unknown azadirachtin content was selected (Table 1). Along with these products, NSKE was also included for assessing its insecticidal potential *vis-à-vis*, CNFs. These products were tested both in the laboratory using leaf dip bioassay method and in the field conditions against DBM.

Neem seed kernel extract

The methodology for home made NSKE has been standardized (Kumar et al. 2000), and is widely recommended (Srinivasan and Krishnamoorthy 1993). The same method of preparation of NSKE was followed. The seeds collected during 2001 fruiting season at Gandhi Krishi Vignana Kendra campus, University of Agricultural Sciences, Bangalore from three individually marked trees (Kumar et al. 2000) were used in both bioassay and field test. The methodology can also be employed by any farmer at the farm level (Kumar et al. 2000).

Indoxacarb 14.5 SC

Indoxacarb belongs to the oxadiazine class of chemistry and blocks the sodium entry into nerve cells, resulting in paralysis and subsequent death of the insect. Manufactured and marketed by DuPont as Avaunt^{TM}, is a formulation with 14.5 % a.i. suspension concentrate (SC). It was used as one of the controls representing the farmers practice.

I. Bioassays

Leaf dip method of bioassay was employed for testing efficacy of different CNFs under laboratory (Jayappa 2000). Three assays were conducted for the same set of CNFs and NSKE at different time intervals during December 2001, February and July 2002.

The test culture

The bioassays were carried out using a laboratory culture of DBM established during August, 1997 (Jayappa 2000). The culture was collected from cabbage plots around Devanahalli and Bangalore in Karnataka, India. The culture was maintained under ordinary room conditions (25.1±2.3ºC) in the laboratory on bold seeded mustard seedlings grown on a vermiculite medium and was expected to have completed around 70 generations by the time the first set of bioassays were carried out during December, 2001. Freshly hatched second instar larvae were used for all bioassays.

Bioassays

Bioassays were carried out using the standard leaf dip method (Matsumura 1975; Jayappa 2000; Kumar et al. 2000). The concentrations for each bioassay were determined after preliminary range finding studies. After identifying the maximum required doses for each bioassay, nine concentrations of the chemical in distilled water and a check were used. For each assay, serial dilutions were made on a semi logarithmic scale using a stock solution of suitable concentration. Each treatment also contained 0.05% soap as the surfactant. Clean mustard leaves were cut to bits of approximately 10 sq cm area with the stalk intact. Each leaf bit was then dipped in the respective concentration of the product for 15 seconds and air dried in shade. The stalk was then wrapped with fresh cotton soaked in clean water and transferred to a petri-plate. Ten freshly hatched second instar larvae of DBM were released on to these leaf bits. Three such replicates were maintained for each concentration of the product. A 0.05% soap solution in distilled water served as the untreated control. In all 300 larvae were used for each assay. The petri plates were maintained in room conditions. Observations were made once in 12 h post treatment, but only the data of 96 h was used (Jayappa 2000) for the probit analysis. The larval mortality or otherwise was ascertained by gently pushing the larvae with a camel hair brush. Moribund larvae that did not respond to pushing were considered as dead. The mortality data were corrected using Abbott's formula and median lethal concentrations were worked out through Probit analysis following Finney (1971).

II. Field Evaluation

Experimental area and the crop

The field experiment was carried out on a farmer's field at Chikka Ankanda Halli, 90 km South of Bangalore. Cabbage crop was raised using the variety Maharani® (MAHYCO Seeds), following standard recommended practices (Anonymous 2000). Nursery was raised under nylon netting without insecticidal treatment and 20 day old hand-picked healthy seedlings were planted.

Planting was done in a randomized complete block design (RCBD) with three replications in a small plot experiment. A spacing of 45 X 45 cm between plants and rows was followed and each plot consisted of six rows with seven plants in each row. A distance of 120 cm was maintained between plots. The crop was grown following the recommended cultivation practices (Anonymous 2000). The crop was harvested on 93 days after planting (DAP).

Treatment imposition

Seven CNFs, NSKE and indoxacarb were the test treatments for the field experiment with an untreated check. Dosages for each of the CNFs were fixed according to the recommendations given by the manufacturers (Table 1). NSKE was however, used at the standard recommended dose of four per cent (Srinivasan and Krishnamoorthy 1993).

The LC50 values of the three sets of assays of CNFs were used to work out their relationship with the declared azadirachtin content in various CNFs. Using the best fit function generated on MS Excel, the azadirachtin value (= insecticidal value) of the NSKE and the Neem Plus were worked out. This data was in turn used to work out the azadirachtin content in the spray solutions, depending on the doses used for different CNFs. Doses used and the resultant expected azadirachtin value of various treatments are given in Table 1.

Table 1: Details of the treatments used in the experiment. The doses of different products used were decided on the basis of the information provided by the manufacturer.

Chemicals	*Manufacturers*	Conc. Aza in formulation	Dose / l	Aza conc. in spray soln.	Revised dose / lt[$]
Limonool®	Bio Multi-tech (Pvt) Ltd., Bangalore	300 ppm	5 ml	1.5 ppm	165ml
Neem Gold®	SPIC Biotechnology Division, Porur, Tamil Nadu	1,500 ppm	4 ml	6.0 ppm	33ml
Econeem®	Margo Bio-control Pvt. Ltd. Bangalore	3,000 ppm	3.25 ml	9.75 ppm	16.5ml
Econeem Plus®	Margo Bio-control Pvt. Ltd., Bangalore	10,000 ppm	1.5 ml	15.0 ppm	4.95ml
Fortune Aza®	Fortune Bio tech Lab, Hyderabad,Andhra Pradesh	30,000 ppm	1.0 ml	30 ppm	1.65ml
NeemAzal-F®	EID Parry (India) Ltd., Chennai	50,000 ppm	0.75 ml	37.5 ppm	0.99ml
Neem Plus®	Bio-Pest Management Pvt. Ltd. Opp. Jakkur Aerodrome, Jakkur Post, Bangalore – 560 064.	*20 ppm**	12 gm	*0.236 ppm*	????
NSKE	Field collected	*1,230 ppm**	40 g	*49.2 ppm*	40g
Avaunt	E.I.DuPont India Pvt. Ltd., 8th Floor, DLF plaza Tower, DLF Quatab Enclave, Phase-I, Gurgaon Haryana – 122 002	14.5 SC	0.4 ml	0.006 % a.i.**	
Untreated Check		---------	--------	--------	

* Kill equivalent expressed as ppm of Aza on the basis of bioassay. ; ** Indoxacarb ;
$ Revised doses are for matching NSKE 4 % in terms of Azadirachtin content in the spray solution

All treatments were imposed by using a high volume knapsack sprayer six times during the crop growth period at 12, 22, 35, 45, 59 and 74 DAP. Maxivet™ at the rate of 1 ml per liter of spray solution was used as a surfactant in all treatments. Water with Maxivet™ served as the untreated check. At the time of imposing the treatments, the amount of spray solution required to spray a plot was calculated each time by spraying water to a set of non experimental plots. All the treatments were then imposed using the same quantity of water.

Observations

Observations were recorded on the incidence of DBM approximately at five day intervals on 12, 17, 22, 28, 33, 40, 45, 54, 59, 64, 72, 79, 84 and 90 DAP. Number of larvae and pupae were counted together on five randomly selected plants in each plot and the mean density of DBM per plant in each plot was calculated. Further as an overall index of the density of DBM, mean number of DBM per day per plant was computed. For this purpose, counts of DBM on the day of planting were considered as zero. Differences between the successive dates of observation were then added up and divided by the crop growth period (90 days, the last day of observation).

Number of opened leaves and leaf area of opened leaves were measured for three randomly selected plants in each plot on 23, 59 and 79 DAP. Head formation was observed in all the treatments starting from 35 DAP. Head size was measured as diameter (in cm) on 64, 79 and 93 DAP for three randomly selected plants in each plot. For these observations, the plants that did not form the head were ignored. Crop was harvested at 93 DAP. At harvest, observations were recorded on the head weight, number of harvestable heads and total yield per plot.

Statistical analyses

Analyses of variance (ANOVA) were carried out on all the data recorded from field experiment. DBM counts were normalized using square-root ($\sqrt{x}$ + 0.25) transformation. Similarly data on per cent harvestable heads per plot were angular transformed. Post hoc comparison of means was done through Tukey's HSD test. Regression analyses were carried out wherever necessary using MS excel

RESULTS and DISCUSSION

Median Lethal Mortality

Bioassays revealed that the LC_{50}s varied greatly for the seven CNFs and the NSKE (Fig. 1). The three bioassays conducted during different time intervals indicated that the LC_{50} values tended to increase with duration of storage for all the CNFs and the NSKE. The LC_{50} values tended to decrease with the concentration of azadirachtin in the product. NSKE however recorded a relatively higher LC_{50} value than other products. Neem Plus with unknown azadirachtin content recorded the highest LC_{50} value.

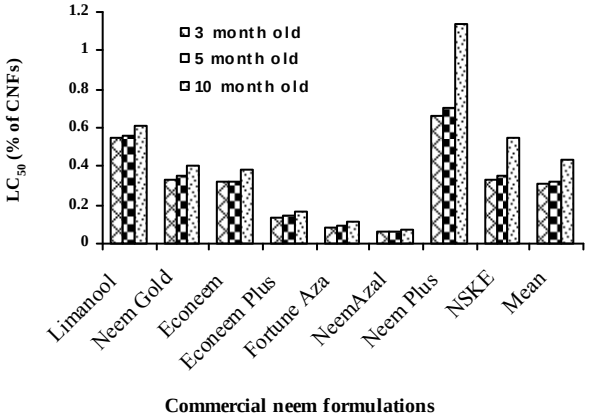

Figure 1. Median lethal values for the seven commercial neem formulations and crude aqueous extract of neem seed kernels against second instar larvae of *Plutella xylostella*, by leaf dip method

An examination of the relationship between the LC_{50} values and the concentration of azadirachtin in the CNFs revealed a strong non-linear relationship (R^2=0.95; n=18; p<0.01). Azadirachtin being the active ingredient in the CNFs, a strong linear relationship was anticipated. However, the observed relationship was not found to be linear and followed a power function, $y=8.588x^{-0.44}$, where 'y' represented the median lethal concentration and 'x' the azadirachtin content. Nevertheless, the strong relationship observed allowed the possibility of estimating the azadirachtin content, as the equivalent kill value, in the unknown products, NSKE and the Neem Plus. The extrapolation from the equation, indicated the azadirachtin content in the neem seeds to be 1230 ppm and in the Neem Plus to be 20 ppm.

Field Experiment

Occurrence of DBM

The infestation of DBM was found throughout the crop growth period and the peak larval numbers were observed around 70 DAP. In general, untreated check plots recorded the highest numbers of DBM on most of the 14 days of observation made at different DAP. The observed trend in the abundance of DBM coincided with earlier observations by Nagarkatti and Jayanth (1982) around Bangalore.

Six applications of the test chemicals resulted in considerable variation in the number of DBM on cabbage at different dates of observation. In general, the 14 observations on the DBM counts per plant suggested a similar trend in the occurrence pattern of DBM on the seven CNFs. The untreated check plots, NSKE and indoxacarb treated plots indicated the maximum deviation in the insect counts from the CNFs. Untreated check plots recorded almost always the highest number of DBM per plant. NSKE and indoxacarb treated plots, however, registered the lowest DBM counts throughout the cropping period (Fig. 2).

During the first three days of observation (12, 17, 22 DAP), the insect counts were observed to be similar in all the treatments ($p > 0.05$). From the 10th day after the first treatment, the treatment effects became more pronounced and significant differences could be observed among the various treatments ($p < 0.05$). Throughout the cropping period, untreated check plots registered the highest DBM counts. In general on most occasions, the Neem Plus, and Econeem registered the closest values, and were generally on par with the untreated plots. Limonool, Econeem Plus and Neem Gold followed these treatments. The next best treatments, in general were Fortune Aza and NeemAzal in that order. NSKE and Indoxacarb treated plots were observed to be the best and the plots receiving these treatments almost always registered an average DBM count of less than one larva per plant.

A further analysis of the overall mean insect counts per day per plant indicated the differences among the treatments to be highly significant ($F_{9,29}=147.18$; $p<0.01$). Post-hoc test revealed six different categories among the treatments. The best treatments were the indoxacarb (0.33±0.01) and the NSKE (0.51±0.10), which were on par with each other and superior to all other treatments. Untreated check registered the highest insect counts per day per plant (2.94±0.46) and was below par to all other treatments.

The study thus clearly demonstrated the superiority of NSKE at 4% in reducing DBM counts on cabbage. NSKE seems to have a long standing life than most other chemicals used against cabbage, and validates the continued recommendation of its use against DBM in cabbage (Srinivasan and Krishnamoorthy 1993; Anonymous 2000). However, the study also revealed the good performance of indoxacarb at 0.006 per cent a.i. On absolute counts the indoxacarb was found superior to NSKE on two occasions, but the differences were not significant. The present study validates the use of indoxacarb in cabbage system by the farmers of South India.

Plant features

Efforts were made to record some of the plant features in various treatments.

Mean leaf number and mean leaf area

Numbers of leaves per plant increased steadily up to 60 DAP in almost all the treatments. From 60 DAP onwards the number of green leaves appeared to reduce up to the harvest time. The various treatments did not differ in their mean number of active open green leaves on all days of observation ($p>0.05$). A monotonous increase in the mean leaf area was observed with the age of the crop. Similar to the leaf number individual treatments were not observed to vary in the mean leaf area ($F_{9,29}=1.75$@12DAP;1.13@35DAP; 0.897@59DAP; 0.747@79DAP; $p>0.05$ for all).

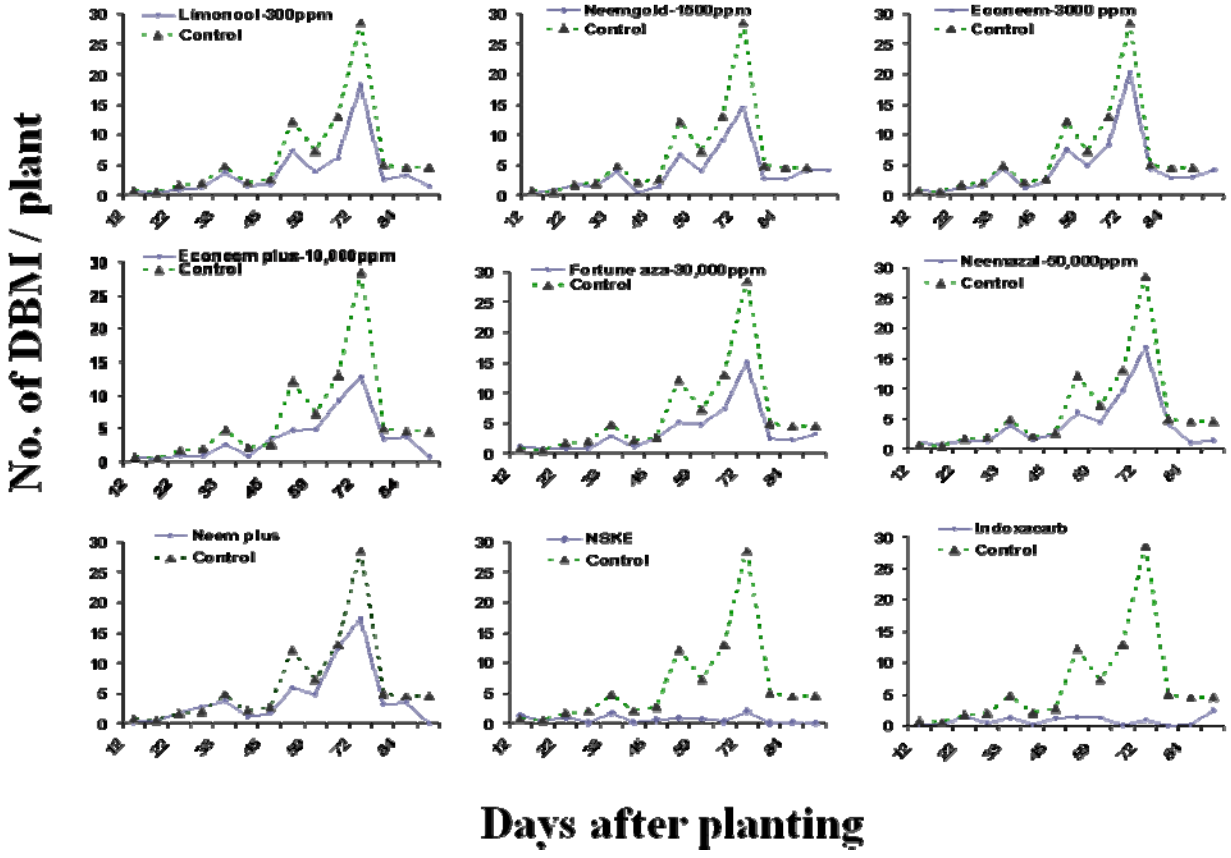

Figure 2. Occurrence of DBM in plots treated with different chemicals on the 14 days of observations. Note the low levels of DBM counts per plant in NSKE and indoxacarb treated plots. The dashed line indicates the DBM counts in untreated check plots.

The above results indicate that the plants in different treatments were not observed to be affected by the incidence of DBM in respect of both the leaf number and the mean leaf area. DBM being a defoliator, it was anticipated that some measurable impact would be observed due to variation in the number of DBM per plant, which varied significantly among the various treatments. However, the extent of damage due to DBM was not measured in terms of the area of leaf lost. Probably as a consequence, the impact in terms of changes in the active leaf area could not be detected. It is possible that the affected treatments have a tendency to compensate for the damage, or that the plant growth pattern is not greatly affected in terms of the leaf number or the mean leaf area within the limits of the observed densities of the DBM in various treatments.

One of the important impacts of feeding damage by DBM is to cause holes in the leaf. This essentially reduces the effective green leaf area for further growth of the plant. This aspect of the exact area lost due to the feeding by the DBM was not monitored in the present study, as we hoped to pick up the difference in terms of the total area itself. Therefore, the exercise indicates the inability of this method to pick up the impact of damage to cabbage by DBM. Consequently, more detailed studies in terms of how the active leaf area changes at different intensities of the DBM on cabbage are required to understand the actual impact.

Growth and the diameter of head

Observations made four times during the crop growth period on the head size indicated the formation of head beginning from the 35 DAP. From then on, there was a continuous increase in the size of cabbage heads measured as mean head diameter in all the treatments. However, the various treatments did not show any significant differences in the mean head sizes of the plants that formed the head at 59 DAP. But the differences were significant at 79 ($F_{9,29}$=9.33; $p< 0.05$) and at the time of harvest at 93 DAP ($F_{9,29}$=11.83; $p<0.01$). At 93 DAP, post-hoc Tukey's HSD test indicated four possible groups with indoxacarb registering the highest head sizes (20.60±1.11 cm) which was on par with NeemAzal and NSKE. Untreated check recorded the least mean head size and was on par with five other treatments (Table 2).

This result was of significance, as despite the lack of observable differences in the mean leaf number and the mean leaf area, strong differences among the treatments were evident in the pattern in which the head growth occurred among the plants that formed the head. Thus the results indicate a possible impact of the DBM counts in various treatments influencing the way the growth of cabbage heads took place in different treatments.

Mean head weight

Mean head weights recorded at harvest varied greatly from a low of 2.61 to 3.64 kg in different treatments (Table 2). Indoxacarb recorded the highest mean head weight, and was significantly superior to all other treatments ($F_{9,29}$=14.29; $p<0.01$). Tukey's HSD indicated three possible categories with respect to mean head weight. Next best treatment was the NeemAzal, which was on par with six other treatments including NSKE. The last category included the untreated check plots, which recorded the lowest mean head weight, and was on par with seven other treatments.

These results indicate a possible additional impact of the DBM counts on the growth of cabbage.

Percent harvestable heads

Considerable variation was found in the per cent harvestable heads recorded at the time of harvest in different treatments (Table 2). The observed differences were significant among the various treatments ($F_{9,29}$=16.61; $p<0.01$). Four categories could be identified on the basis of the Tukey's HSD. The NSKE treatment recorded the highest mean per cent head formation (94.45%). This was also superior to all other treatments it was followed by indoxacarb treatment, which was on par with five other treatments. Least head weight was recorded in the Neem Gold treated plots which was on par with six other treatments including the untreated check that recorded the second lowest head weight. Thus the most important impact of the DBM in cabbage was in reducing the per cent harvestable heads in different treatments. The impact of this effect is expected to have a direct bearing on the total plot yield.

Table 2: Different yield parameters observed at 93 DAP during harvest of the cabbage at Chikka Ankanda Halli during summer 2001

Treatments	Head diameter (cm) (Mean±sd)	Head weight in kg (Mean±sd)	%harvestable heads (Mean±sd)	Yield (kgs)/plot (Mean±sd)
Limonool	18.83 ± 0.91	2.63 ± 0.10	61.11 ± 15.85	67.62 ±19.21
Neem Gold	18.77 ± 0.65	2.73 ± 0.36	55.56 ± 5.99	63.65 ±10.70
Econeem	17.67 ± 1.63	2.70 ± 0.39	70.63 ± 13.54	79.27±12.83
Econeem Plus	18.13 ± 1.88	2.83 ± 0.88	70.63 ± 13.11	73.95 ± 21.43
Fortune Aza	19.40 ± 1.25	2.89 ± 0.54	65.08 ± 16.89	78.18 ± 20.60
NeemAzal	19.33 ± 1.50	3.12 ± 0.53	76.19 ± 10.91	99.34 ± 17.90
Neem Plus	18.27 ± 1.38	2.82 ± 0.39	66.67 ± 2.38	78.78 ± 9.45
NSKE	19.47 ± 1.01	2.77 ± 0.26	94.44± 1.37	109.70 ± 9.66
Indoxacarb	20.20 ± 1.11	3.64 ± 0.56	80.95 ± 4.12	123.33±14.03
Control	17.53 ± 0.50	2.61 ± 0.24	60.32 ±16.21	56.39 ± 3.05

Yield of cabbage heads per plot

Considerable range in mean plot yield was observed in different treatments ranging from as low as 56.39 in untreated check plots to 123.33 kg per plot in indoxacarb treated plots. The differences among the various treatments was significant ($F_{9,29}$=19.12; p< 0.01) and the post-hoc test revealed three groups. Indoxacarb, NSKE and the Neem Azal treated plots were the best, and were on par with each other. The second group consisted of Neem Azal, Econeem and the Neem Plus treated plots. The latter two, however were also on par with all other treatments that formed the third group (Table 2).

These results clearly indicate the superiority of indoxacarb as the best treatment followed by NSKE in improving the yield of cabbage heads. But the question remains as to how far these results are related to the relative differences in the impact of treatments on the counts of DBM. In order to understand this aspect, attempts were made to correlate the various yield parameters with the mean DBM counts observed in different plots.

Interrelationships between the yield parameters

An attempt made to understand the interrelationship between the various yield parameters revealed that many of the parameters considered were strongly related to one another (Table 3). Only the number of leaves per plant was observed to be uncorrelated with all the other plant parameters considered except the mean leaf area. Mean leaf area, further, did not show any relationship with per cent harvestable heads.

Table 3. Inter-correlation matrix for the different yield parameters observed in cabbage at Chikka Ankanda Halli during 2001

Parameters	No. of Leaves	Leaf Area	Head Diameter	Head Weight	Percent Harvestable Heads	Yield
Mean DBM Counts	.027^{NS}	-.293^{NS}	-.677**	-.584**	-.661**	-.735**
# Leaves		-.604**	-.078^{NS}	-.090^{NS}	-.292^{NS}	-.212^{NS}
Leaf Area			.376*	.488**	.328^{NS}	.516**
Head Diameter				.782**	.464*	.776**
Head Wt.					.388*	.764**
% Harvestable Heads						.798**

** Correlation is significant at the 0.01 level
* Correlation is significant at the 0.05 level
NS : p>0.05

A strong negative relationship observed between the leaf number and the leaf area may indicate a mechanism of compensation to damage by the plant. However, more detailed studies are required to understand this phenomenon.

Relationship between the DBM counts and the yield parameters

Considering that the different yield parameters are by and large interrelated, it would be of interest to understand the relationship between the insect counts and various yield parameters of cabbage. Therefore, the mean DBM counts per day per plant recorded for each plot was then correlated with various yield parameters. It was observed that the mean DBM counts were strongly correlated with the mean head diameter, mean head weight, per cent harvestable heads per plot and the plot yield (Table 3). The leaf characteristics, the mean number per plant and the leaf area were not found to be correlated with the mean DBM counts. The results of the study thus overwhelmingly suggest that the impact of DBM to be the major factor responsible for the observed yield and the related parameters in cabbage.

Is NSKE superior to other treatments? The cost factor

Two important aspects were evident from the study in respect of NSKE. One is the high impact on insect counts and the second most important factor was the consequent increase in the overall yield of cabbage. Indoxacarb was found superior to NSKE in increasing the yield. Therefore, the question arises as to whether NSKE can continue to be recommended as the ideal management technique against DBM in cabbage. In order to understand this aspect, an attempt was made to work out the cost factor and the marginal returns observed in different treatments. The mean Indian Rupees (1 = US$ 0.02) invested per plot was lowest for the NSKE (INR 8.32). Costs of most other treatments remained more or less on par, except those of Econeem Plus (INR 14.69), Fortune Aza (INR 16.90) and Neem Plus (INR 10.92). Further, as the market prices of cabbage heads can vary greatly from INR 0.50 to 8.00 within days, an attempt was made to avoid the profit factor and the yield realized per unit of rupee invested was worked out. Except Neem Gold, all other commercial neem formulations recorded positive marginal returns. However, the results indicated an enormous advantage for NSKE which was followed by indoxacarb (INR 20.80). These results thus strongly suggest the importance of use of crude extracts of neem. This leaves us with the question of what limits the better performance of commercial neem formulations.

Limiting factor for the commercial neem formulations

Commercial neem formulations are expected to serve as ready to use chemicals that have the potential to replace NSKE that is cumbersome to prepare. Commercial formulations are also expected to guarantee a quality assurance over crude extracts which can vary 3 to 5 folds from tree to tree (Senrayan 1998; Jayappa 2000; Kumar et al. 2000; Ashoka and Kumar 2002). But the study revealed all the commercial neem formulations to perform below par to crude extracts under field conditions in all aspects of the crop growth. They were found to be inferior to NSKE in both reducing the DBM counts on cabbage and also in failing to enhance the plant performance with respect to various yield parameters considered, at the recommended doses.

The study used recommended doses of the formulations for preparation of spray mixture. These doses, however, resulted in variable content of azadirachtin in the spray mixtures. All the formulations basically use azadirachtin as the major active ingredient. As a consequence, it is possible that this variation in the azadirachtin content has a significant role to play in the performance of various formulations. Therefore, we checked the relationship between the azadirachtin content in the spray mixtures and the mean DBM counts in different neem based treatments. Results indicated a strong relationship between the azadirachtin content in the spray solution and the mean DBM counts per day per plant (Fig. 3a). The best fit function was an exponential function ($r=0.699$; $p<0.01$) suggesting that the DBM counts are related to the quantity of azadirachtin used in the spray solution. Further, the azadirachtin content in the spray solution also directly influenced the yield per plot (Fig. 3b; $r=0.796$).

The crude aqueous NSKE at four per cent accounted for an estimated 49.20 ppm of azadiarchtin in the spray mixture and none of the neem formulations matched NSKE in the content of azadirachtin in the spray mixture in the first place. Secondly, their performance against DBM in cabbage was below par to NSKE. The results thus identify the doses recommended as the great limiting factor for improved performance of the neem formulations and suitable modifications to enhance the azadirachtin content in the spray mixture should facilitate the improved performance of commercial neem formulations. This also leads to the question of what should be the extent of modification. It was observed that 4% NSKE has 49.20 ppm of azadirachtin in the spray solution. To achieve an equivalent concentration of azadirachtin in the spray solutions of CNFs, very huge changes are to be made in the recommendations of doses for some of the formulations (Table 1). The changes apparently followed a trend. Changes required in the dose recommendations are inversely related to the azadirachtin content in the formulations. With a current general cost factor of 2:1 between the formulations and the NSKE, the formulations with lower doses do not have a chance for this modification. Therefore, in the long run, cost effective azadiarchtin rich formulations seem to have a better future from the point of view of field utility; although, the use efficiency was previously shown to decrease with increasing azadirachtin content in the CNFs (Kumar et al. 2003).

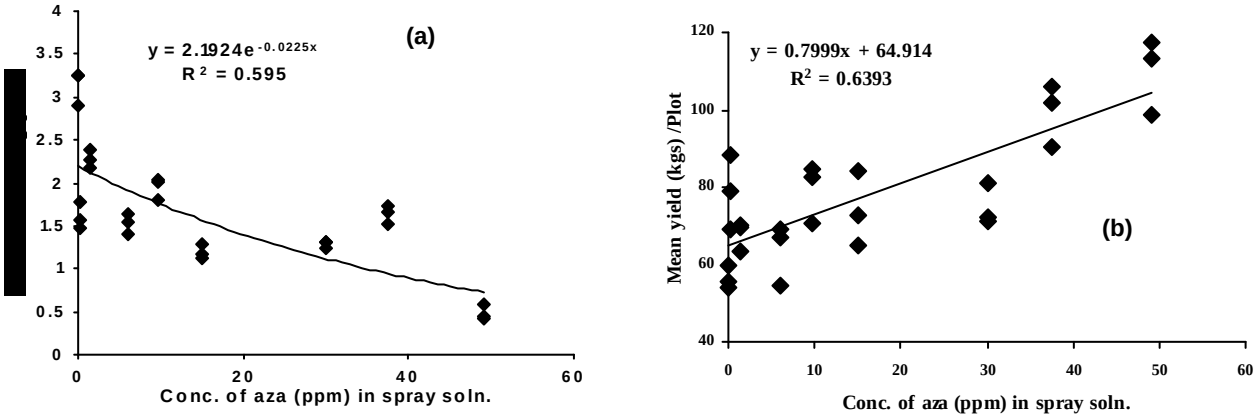

Figure 3. Relation between the aza content in spray solution of different treatments to the DBM counts per plant per day (a) and to the yield of head cabbage in kg per plot (b). Note the non-linear nature of the relationship

How good are the best treatments?

The study established NSKE and the indoxacarb as the best treatments for the management of DBM in cabbage, as both these treatments greatly reduced the mean DBM counts and contributed for increased yields. But both the treatments were observed to pose some problems. Indoxacarb, was found to affect the development of the head in cabbage. As much as close to 20 per cent of the plants did not develop heads. Among these, nearly fifty per cent developed in to miniature leafy plants with reduced high density of leaves. The symptoms were similar to a phyllody. Such plants were not detected in any of the other treatments including the untreated check. It is possible that indoxacrab at high doses might cause such a symptom and accidental increased doses while spraying might have contributed for such a phenomenon. It is also possible that the higher doses at some specific stage of the crop could have caused such a growth in cabbage. It might also be necessary to check whether the observed feature is a specific variety-chemical reaction. Further studies are therefore necessary to account for the observed malformation in indoxacarb treated cabbage plants. One other specific observation was in respect of the high aphid populations in indoxacarb treated plots. Obviously, indoxacarb did not check the aphid build up. But for these limitations, the indoxacarb treatment at the doses used appears to be good for managing DBM in cabbage system.

NSKE posed a major problem in cabbage. Despite recording very low pest incidence throughout the crop growth period and also a very high per cent harvestable heads, the plot yields were not commensurate with these features. It was observed that the mean head weight in NSKE treated plots was considerably low, and was below par to the best of the treatments. NSKE treated plots were also more greenish than the plots receiving other treatments. Thus, as indicated earlier (Schmutterer 1992), the NSKE might affect the wax coating on the leaves and caused the reduced weight gain in cabbage heads. The best of the treatment registered as much as 3.64 kg per head while NSKE treated plots recorded only 2.77 kg. Clearly this low head weight nullified the advantages gained in terms of reduced DBM counts and increased percent harvestable heads. Therefore, it would be ideal to explore this aspect further to identify the causal factor responsible and develop mechanisms to overcome these limitations. In specific, critical stages of the crop and the concentrations of NSKE might need re-evaluation.

CONCLUSION

NSKE and indoxacarb treated plots maintained low levels of DBM populations and registered higher yield per plot than all other CNFs. Failure of the CNFs at the recommended doses in managing the DBM populations may be due to the lower azadirachtin content per unit of spray volume compared to NSKE. Attempts to increase the doses of azadirachtin in the spray solutions with CNFs to match NSKE will only make them more expensive for field use. Thus, NSKE at 4% is most economical and safer for the management of DBM in cabbage system.

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Field assessment of aqueous suspension of *Zoophthora radicans* conidia and low application rates of imidacloprid for control of diamondback moth, *Plutella xylostella* on cauliflower in Tamil Nadu, India

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ABSTRACT
In small-scale field trials, aqueous suspension of *Zoophthora radicans* conidia and its combinations with low rate of imidacloprid were evaluated against diamondback moth (DBM), *Plutella xylostella* larvae on cauliflower in Palladam (Trial 1) and Coimbatore (Trial 2) of Tamil Nadu state, India. The trials were arranged in a randomized complete block design having four replications for each treatment. In trial 1, cauliflower plots were sprayed twice with doses containing $2.0X10^7$, $1.0X10^7$ and $6.7X10^6$ conidia/ml of *Z. radicans* alone and combinations with imidacloprid 17.8 SL (50 ml/ha) at a 14-day interval. In trial 2, the cauliflower plots were sprayed twice with high rate of aqueous suspension of *Z. radicans* at $1.7X10^{13}$ conidia/ha alone and together with imidacloprid 17.8 SL at the rate of 50 ml/ha. All treatments significantly reduced DBM densities in treated plots compared to the control plots. In trial 1, during the 10-day period of larval sampling, an overall mean larval density estimated from control plots (7.25 larvae/plant) was significantly higher than those from plots sprayed with the three aqueous dilutions of *Z. radicans* (5.0-5.5 larvae/plant) and *Z. radicans* + imidacloprid (3.25-4.25 larvae/plant). *Z. radicans* at higher rate was only 33.30% efficient in trial 1 and 28.00% in trial 2. The high-rate of *Z. radicans* conidia plus low rate of imidacloprid resulted in the most significant DBM control, yielding an overall mean efficacy of 69.23% in trial 1 and 72.00% in trial 2. The low rate spray of imidacloprid alone in trial 2 was less efficient recording an efficacy of 33.00-56.00%). Aqueous suspension of *Z. radicans* together with imidacloprid 17.8 SL at a lower rate (50ml/ha as against its recommended rate of 350 ml/ha) could be incorporated into the DBM management system.

KEYWORDS
Zoophthora radicans, *Plutella xylostella*, aqueous suspension, imidacloprid, cauliflower

INTRODUCTION
The diamondback moth (DBM), *Plutella xylostella* L. (Lepidoptera: Plutellidae) is one of the most important pests devastating cruciferous vegetables worldwide. The moth is multivoltine with as many as 6-18 generations per year (Talekar and Shelton 1993). The pest infests the plant at all the growth stages causing defoliation and stunting of plant growth. Outbreaks of DBM in Southeast Asia sometimes cause crop losses of more than 90 per cent (Verkerk and Wright, 1996). Severe infestation may result in 100% yield loss in endemic areas (Lingappa *et al.* 2000). Various methods are available to control this pest. However, control of DBM is largely practiced by applying various synthetic chemical insecticides as frequently as ten times over a growing season or even more frequently (Regupathy, 1996), which not only affects safety due to high toxic residues in produces, but also led to control failures owing to significant resistance to almost every insecticide applied in the field including new chemistries such as spinosads, avermectins, neonicotinoids, pyrazoles and oxadiazones (Sarfraz *et al.*, 2005). The moth is a particularly severe pest of cauliflower in Tamil Nadu State of Southern India (Regupathy, 1996), where rapid development of resistance to all major classes of insecticides has rendered it a problem unmanageable by conventional insecticidal use.

Currently, an insecticide commonly recommended for use in DBM control is imidacloprid 17.8 SL at an application rate of 300-350 ml/ha. However, since resistance to chemical insecticides can occur, it is imperative to avoid over reliance on the widely used insecticides. Alternative approaches to the conventional use of synthetic insecticides for the control of DBM are highly warranted because of increasing importance of integrated management strategies relying on biocontrol possibly combined with selective insecticide application. Fungal entomopathogens are considered the most promising alternative approach because they are currently being developed as biocontrol agents of many insect pests including *P. xylostella* (Pell and Wilding 1992; Selman *et al.*, 1997; Toshio, 2000). The entomophthoralean fungus, *Zoophthora radicans* Brefeld (Batko) is a virulent biocontrol agent which

regularly causes epizootics in populations of DBM in cabbage and cauliflower in Southern India (Gopalakrishnan *et al.*, 1999) under the most favourable conditions. The infection by *Z. radicans* starts when the primary or capillary conidia that are forcibly discharged from conidiophores land on the cuticle of a suitable host insect (Pell and Wilding 1992). When the propagules come in contact with the insect cuticle, they germinate and grow directly through the cuticle to the inner body of their host even without being consumed and proliferate throughout the host's body producing toxins (Galaini-Wraight *et al.*, 1992), and eventually killing it (Hajek and St. Leger, 1994; Furlong *et al.* 1995; Furlong and Pell 1996; Sarfraz *et al.* 2005). Once the fungus has killed its host, it grows out through the softer portions of the cuticle, covering the cadaver with a layer of creamy-white fluffy fungus growth.

Previous research has demonstrated a high potential of *Z. radicans* for biological control of DBM (Furlong and Pell, 1996), and can be mass produced and formulated for field use (Wraight *et al.*, 2003). However, the slow mode of action of the fungus had led to criticism for its use for field control of the pest. Tandem application of fungal entomopathogens with chemical insecticides may also be used to prevent or manage resistance problems in insect pests (Rodriguez and Peck, 2009). Several studies have proven that sublethal doses of a number of insecticidal nitroguanidine compounds including imidacloprid, increased susceptibility of a number of insect pests to various fungal entomopathogens. The objective of this study was to determine whether low rate of imidacloprid could enhance the efficacy of *Z. radicans* on field populations of DBM larvae, and to investigate the feasibility of incorporating *Z. radicans* into DBM management strategies.

MATERIALS AND METHODS

Source of fungal pathogen

An isolate of *Zoophthora radicans* (ZrCm KKL 1194), was originally collected and isolated from rice leaf-folder, *Cnaphalocrocis medinalis* Guenee (Pyraustidae: Lepidotera) in November 1994 from wetland rice fields of Experimental Research Farm (ERF) in Karaikal of Puducherry Union Territory, India (Ambethgar, 1995). The fungus culture was maintained at very low temperature and rarely sub-cultured on Sabouraud dextrose agar (SDA). We reactivated the isolate through larvae *of C. medinalis* and conidia were used to infect *P. xylostella* larvae, and subsequently reisolated from mycosed cadavers. This reisolated strain was passed thorough serial infection in *P. xylostella* larvae and re-isolation process was repeated for another 4-5 times, at which time the strain was highly adapted to this host (Ambethgar, 2002). The reisolated strain was routinely grown on SEMA (80% Sabouraud dextrose agar, 11.5% fresh milk, 8.5% egg yolk in 1 l distilled water) at 10°C in a photoperiod of L12 : D12, and sub-cultured every three months as described by Furlong *et al* (1995).

Preparation of aqueous suspension

An aqueous suspension containing 5% emulsifier, 0.3% suspension stabilizer (sodium carboxymethyl cellulose), and 0.1% UV protectant (Feng *et al.*, 2004) was used to formulate *Z. radicans* into an emulsifiable suspension. *Z. radicans* preparation containing imidacloprid (Zr+Im) were obtained by adding imidacloprid 17.8 SL to *Z. radicans* at the ratio of 1% (v/v). Prior studies have proved that the chemical is biologically compatible with *Beauveria bassiana* (Xu *et al.*, 2002), and may synergistically interact with other fungal formulations (Quintela and McCoy, 1998; Furlong and Groden, 2001).

Trial location and layout

Field trials were performed at Palladam, Coimbatore, Tamil Nadu from December-February (Trial 1), and in Udumalpet, Coimbatore, Tamil Nadu from December-February (Trial 2). Both field sites were previously endemic for *P. xylostella* infestation in every growing season. The cauliflower crop cv Ooty-1 was raised in nursery and transplanted after one month. Two parallel rows of 3-week old cauliflower (*Brassica oleracea* var *botrytis*) seedlings were transplanted at a spacing of 40 X 40 cm. The trials were laid out in a randomized complete block design with four replications for each treatment having a plot size of each 8-m X 5-m. Data collection commenced three weeks after transplanting.

Trial 1

The trial 1 attempted to determine an effective application rate for the *Z. radicans*. The fungal treatments included 500-, 1000- and 1500- fold aqueous dilutions ($2.0X10^7$, $1.0X10^7$ and $6.7X10^6$ conidia/ml) of *Z. radicans*, and *Z. radicans* + imidacloprid 17.8 SL @ 50 ml/ha. Spray suspensions were prepared just before field application. The sprays were initiated 30 days after transplanting (DAT). Each replication was sprayed twice in the evening hours (17-18 h) before sunset using a high volume knapsack hydraulic sprayer at a spray fluid volume of 500L ha^{-1}. The three aqueous dilutions sprayed each time corresponded to the application rates of $2.0X10^7$, $1.0X10^7$ and $6.7X10^6$ conidia/ml for *Z. radicans* alone, and combinations of the same conidial rates with imidacloprid 17.8 SL at the rate of 50.0 ml/ha. A 1-m wide buffer area around the field and a 1-m wide area between every plots were not sprayed to prevent the drift effects.

Trial 2

Since the best treatment found in trial 1 was a combined spray of 500-fold dilution of *Z. radicans* and imidacloprid 17.8 SL at the rate of 50ml/ha, the trial 2 included only four treatments *viz.*, 500-fold dilution aqueous dilutions of *Z. radicans*, *Z. radicans* + imidacloprid @ 50 ml/ha, 50 ml of imidacloprid 17.8 SL alone and a water spray control, with each being replicated for four times in 40-m^2 plots. All replicated plots on the field were arranged in a randomized complete block design, and sprayed twice at 14-d interval using a high volume knapsack hydraulic sprayer with a small nozzle of 0.9 mm. Each plot received 5 l aqueous dilution with the application rate from each spray was $1.7X10^{13}$ conidia/ha for *Z. radicans* alone, the

same conidial rate plus imidacloprid 17.8 SL at 50 ml/ha for *Z. radicans* + imidacloprid, and 50 ml/ha imidacloprid alone.

Data collection and analysis

Pest monitoring commenced three weeks after transplanting and continued thereafter until the harvest. Treatment application commenced one day after pre-treatment data collection. Initial counts of larvae of the *P. xylostella* (number of larvae/plant) were made *in situ* the day before the first spray from five plants arbitrarily sampled from each plot with each sample consisting of five leaves. Post-treatment sampling was conducted for larval counts up to 10 d at intervals of 5 d using the same protocol. The percent decrease of *P. xylostella* larval density (D) and the efficacy (E) of each treatment for DBM reduction relative to control were used as indices of control efficacy and computed following Feng *et al* (2004) with minor modifications.

$$D = \frac{d_{T0} - d_{T1}}{d_{T0}} \times 100$$

$$E = \left(1 - \frac{D_{CK0} \times D_{T1}}{D_{CK1} \times D_{T0}}\right) \times 100$$

Where D_{T0} and D_{T1} were mean larval densities (number of larvae per plant) estimated for a given treatment before and after the first spray; D_{CK0} and D_{CK1} were mean larval densities from control plots before and after the first spray. Variances of larval counts among the treatments and the sample days were determined using the procedure for analysis of variance (ANOVA) in AGRISTAT (v.6. 2003), and ANOVA was used to compare pest infestation among the treatments and means were separated using DMRT.

RESULTS AND DISCUSSION

Trial 1

Trends in larval density

The result of trial 1 is shown in Table1. The effects on the pest populations varied among the treatments compared to the untreated plots. The larval counts made the day before the first spraying (1 DBS) ranged between 5.5 and 7.0 larvae/plant which did not differ significantly among the treatments, but exceeding an economic damage level of two larvae per plant (Sarfraz *et al.,* 2005). However, the overall post treatment estimates on the *P. xylostella* larval densities were significantly different among the treatments, and among the sampling days. Compared to the estimates from control plots, the DBM larval densities were reduced significantly by the first spraying of all *Z. radicans* + imidacloprid dilutions on 5-day of larval sampling. During the 10-day period of larval sampling, an overall mean larval density estimated from control plots (7.25 larvae/plant) was significantly larger than those from plots sprayed with the three aqueous dilutions of *Z. radicans* (5.0-5.5) and *Z. radicans* + imidacloprid (3.25-4.25).

The second spraying also maintained similar trend for another 10 days. The mean larval density estimated from control plots (5.25 larvae/plant) was significantly higher than those from plots sprayed with the three aqueous dilutions of *Z. radicans* (3.5-4.0) and *Z. radicans* + imidacloprid (2.0-2.5). Of those treatments, 500-fold dilution of *Z. radicans* + imidacloprid was consistently best in suppressing the DBM larval population throughout the trial. This indicates that two sprayings of *Z. radicans* + imidacloprid dilutions did produce an effect on the population decline through the whole population tended to decrease during the trial.

Control efficacy

In trial 1, DBM larval densities in *Z. radicans* and *Z. radicans* + imidacloprid treatments decreased by 14.8-40.7% more than the natural decline of the pest population in control plots on day 5 and 24.1-51.1% more on day 10 respectively, after first spraying. Generally, the *Z. radicans* + imidacloprid treatments yielded higher efficacy than *Z. radicans* alone. The same trend was also witnessed during second spraying. A significant difference was detected among the three *Z. radicans*+ low application rates of imidacloprid, but not in *Z. radicans* alone. The best efficacy of DBM control up to 69.23% resulted from the low application rate of *Z. radicans* + imidacloprid after 5 days of second spraying.

Trial 2

Trends in larval density

The results of trial 2 are given in Table 2. The initial mean larval counts made on 1 DBS, ranged between 5.0 and 6.0 larvae per plant with insignificant difference among the treatments. The trend of DBM larval population in control plots tended to increase throughout the trial period. During first spraying, the mean larval density differed among the treatments and sampling days, being lower in Z. radicans + imidacloprid (4.75 larvae per plant) and imidacloprid (2.75 larvae per plant) than Z. radicans alone (5.75 larvae per plant) and in control (6.0 larvae/plant). During second spraying also similar trends of reduced larval density witnessed.

Control efficacy

In trial 2, the DBM densities in the treatments of Z. radicans, Z. radicans + imidacloprid, and imidacloprid alone decreased by 4.17, 54.17 and 20.83% respectively on day 5, and 16, 72 and 44% respectively on day 10 after first spraying. After second spraying, the decline in DBM population compared to control was 29.63% for Z. radicans alone, 85.19% for Z. radicans + imidacloprid and 51.85% for imidacloprid alone. The estimates of both per cent density decrease and relative efficacy indicates that two sprayings of 500-fold Z. radicans + imidacloprid dilution apparently controlled the DBM population more effectively than the other two

treatments. It is evident that DBM control was less satisfactory when fungal preparation was applied alone.

Interaction of Z. radicans + imidacloprid

Synergistic interactions of imidacloprid with fungal entomopathogens in insect control have been reported previously (Quintela and McCoy, 1998; Furlong and Groden, 2001). In the present study, more consistent DBM control was achieved at the high rate of *Z. radicans* + low rate of imidacloprid than the same rate of *Z. radicans* alone, suggesting a possible synergistic effect of the low imidacloprid rate with the pathogen. This result is similar to previous report against potato beetles (Zhao *et al.*, 2000), cereal aphids (Vandenberg *et al.*, 2001), whiteflies (Malsam *et al.*, 2002) and tea leafhopper (Feng *et al.*, 2004). Single spray of oil-based preparation of *Beauveria bassiana* at a rate of $1.9X10^{13}$ conidia/ml plus imidacloprid 10% WP (20g/ha) led to reduce >90% aphids (*Mysus persicae*) on cabbage in four-weeks (Ying *et al.*, 2003). Use of selective insecticides at suitable concentrations in favour of germination, mycelial growth, conidial production and survival of insect pathogenic fungi is desirable. Joint application of selected strains of fungal entomopathogen with low doses of insecticides is gaining importance in insecticide resistance management in insect pests as a component of IPM programme.

Several insecticides at sub-lethal concentrations were reported to be compatible with major taxa of fungal entomopathogens such as *B. bassiana, M. anisopliae, P. farinosus, P. fumosoroseus* and *V. lecanii* in different situations (Hiromori and Nishigaki, 2001; Malsam *et.al.*, 2002). Reports of Xu *et al.* (2002) indicated that for practical application, it is important to make use of positive interactions of low residue insecticides with suitable fungal agents, and also possible to select such insecticides biologically compatible with fungi. Synergistic interactions between chemical insecticides and fungal entomopathogens are enormous and well documented (Ambethgar, 2009). It is worth exploring the effects of various new molecule insecticides at sub-lethal doses on entomofungi as two-in-one tank mix strategy to reduce the selection pressure of insecticides and thus avoiding the resistance risks. While doing so, adequate care should also be taken because insecticides at sub-lethal doses may sometimes lead to the resurgence of less important insect pests. In Coimbatore and other high altitude regions of Tamil Nadu, weather is conducive for the use of fungal entomopathogens in growing season except during hot summer season. In conclusion, the present research constitutes the first attempt to investigate into the co-application of *Z. radicans* and low rate of insecticide against the field control of *P. xylostella* in Tamil Nadu, India.

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Table 1. Effects of three aqueous suspensions of *Z. radicans* and their mixtures with imidacloprid for control of *P. xylostella* population in Palladam, Tamil Nadu, India

Treatment		Mean larval population (MLP) and per cent reduction over control (ROC)							
			I Spray				II Spray		
		1 DBS	5 DAS		10 DAS		5 DAS		10 DAS	
			MLP	ROC %	MLP	ROC%	MLP	ROC%	MLP	ROC%
1.	*Z. radicans* 1500x	5.50[a]	5.75[f]	14.81	5.50[f]	24.14	4.50[f]	30.77	4.00[f]	23.61
2.	*Z. radicans* 1000x	6.00[a]	5.50[e]	18.52	5.25[e]	27.59	4.25[e]	34.62	3.75[e]	28.57
3.	*Z. radicans* 500x	5.75[a]	5.25[d]	22.22	5.00[d]	31.03	3.75[d]	42.31	3.50[d]	33.33
4.	*Z. radicans* 1500x + Imidacloprid @ 50ml/ha	6.00[a]	4.50[c]	33.33	4.25[c]	41.38	2.75[c]	57.69	2.50[c]	52.38
5.	*Z. radicans* 1000x + Imidacloprid @ 50ml/ha	6.50[a]	4.25[b]	37.04	3.75[b]	48.28	2.25[b]	65.38	2.25[b]	57.14
6.	*Z. radicans* 500x + Imidacloprid @ 50ml/ha	6.25[a]	4.00[a]	40.74	3.25[a]	51.17	2.00[a]	69.23	2.00[a]	61.90
7.	Water spray control	5.75[a]	6.75[g]	00.00	7.25[g]	00.00	6.50[g]	00.00	5.25[g]	00.00

In a column means followed by similar letters are not significantly different (P=0.05) by DMRT

Table 2. Effects of aqueous suspensions of *Z. radicans* and their mixtures with imidacloprid for the control of *P. xylostella* population in Udumalpet, Tamil Nadu, India

Treatment		Mean larval population (MLP) and per cent reduction over control (ROC)							
			I Spray				II Spray		
		1 DBS	5 DAS		10 DAS		5 DAS		10 DAS	
			MLP	ROC %	MLP	ROC%	MLP	ROC%	MLP	ROC%
1.	*Z. radicans* 500 x	6.00[a]	5.75[c]	04.17	5.25[c]	16.00	4.75[c]	29.63	4.50[c]	28.00
2.	*Z. radicans* 500 x + Imidacloprid @ 50ml/ha	5.25[a]	2.75[a]	54.17	1.75[a]	72.00	1.00[a]	85.19	1.75[a]	72.00
3.	Imidacloprid @ 50ml/ha	5.00[a]	4.75[b]	20.83	3.50[b]	44.00	3.25[b]	51.85	3.00[b]	52.00
4.	Water spray control	5.50[a]	6.00[d]	00.00	6.25[d]	00.00	6.70[d]	00.00	6.25[d]	00.00

In a column means followed by similar letters are not significantly different (P=0.05) by DMR

Effects of sub-lethal doses of *Bacillus thuringiensis* (Bt) δ-endotoxins against natural enemies of diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae)

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ABSTRACT
δ-endotoxins produced by *Bacillus thuringiensis* (Bt), a gram positive, soil-borne bacterium, are highly specific to insect pests, and are used as biopesticides to control several pest species including diamondback moth (DBM). Bt is now widely used in several countries because several crops have been genetically engineered with *cry* genes for the past few decades. Because efforts are underway to develop and introduce insect resistant transgenic brassicas, it is imperative to assess the effects δ-endotoxins on non-target natural enemies of DBM. Effects of Cry1B and Cry1C were evaluated against a generalist predator, *Mallada basalis* and larval parasitoids *Diadegma semiclausum* and *Cotesia plutellae*. The DBM larvae were either fed or treated with three sub-lethal doses (LC_{25}, LC_{50} and LC_{75}) of Cry1B and Cry1C. There was no significant difference among the sub-lethal doses compared with untreated check in larval mortality, pupal mortality, adult emergence, development time, fecundity and egg hatchability of *M. basalis* when it was reared on Cry1B-fed DBM. Similar results were obtained when *M. basalis* was reared on Cry1C toxin treated DBM. However, the adult emergence was significantly lower (72%) at LC_{75}, compared to check (88%). Values for LC_{25} and LC_{50} doses were intermediate and similar to either of the extremes. Although sub-lethal doses of Cry1C did not have any significant effects on *M. basalis*, pupal mortality was significantly higher (25.67%) at LC_{75}, compared with all other treatments when it was reared on Cry1C-fed DBM. When the DBM larvae were fed on the sub-lethal doses of Cry1B and Cry1C toxins, and exposed to the larval parasitoids, the toxins did not adversely affect the parasitoids' pupation, pupal weight, adult emergence and the performance of their F_1 progenies.

Keywords
Bacillus thuringiensis toxins, diamondback moth, predator, parasitoid

INTRODUCTION
Bacillus thuringiensis (Bt) is a gram positive, soil-borne bacterium. It produces insecticidal crystal proteins or δ-endotoxins that are commonly known as Cry toxins that are encoded by *cry* genes. They are highly specific to insect pests, and are used as biopesticides to control several pest species (Lambert and Peferoen, 1992; Schnepf et al., 1998). Bt-based biopesticides constitute about 90% of the global biopesticide market (Rodrigo-Simon et al., 2006), although biopesticides represent only 2% of the total global insecticide market (Lambert and Peferoen, 1992). However, Bt is now widely used in several countries because several crops have been genetically engineered with *cry* genes for the past few decades. As of 2008, insect resistant transgenic crops are being grown in an area of 46 million ha worldwide, which constitutes about 37% of global biotech acreage (James, 2008). Several crops expressing *cry* genes are also under development. Efforts are underway to develop and introduce insect resistant transgenic brassicas either with a single gene (Bai et al., 1993; Bhattacharya et al., 2002; Wang et al., 2003) or with two genes (Paul et al., 2005; Russell et al., 2008).

Bt-based biopesticides have been used for the past several decades and they are not pathogenic to mammals, birds, amphibians, or reptiles, but are very specific to the groups of insects and invertebrate pests against which they have activity (Schnepf et al., 1998). Bt biopesticides are integral components in both organic and integrated pest management systems (Glare and O'Callaghan, 2000). However, effects on non-target natural enemies cannot be ignored, because the insecticidal proteins are continuously produced in high doses in the tissues of transgenic plants engineered with *cry* genes. Hence, the non-target natural enemies could be exposed to toxic proteins through non-susceptible or sub-lethally affected target and non-target phytophagous insects feeding on transgenic plants. In addition, pollinators and decomposers also could be exposed (Jepson et al., 1994; Romeis et al., 2008). This becomes an important pathway for the ecological impact of transgenic crops as these arthropod species fulfill important agroecological functions (Andow and Hilbeck, 2004; Romeis et al., 2008).

Mallada basalis (Walker) (Neuroptera: Chrysopidae) is one of the two species studied in detail as a predator in the genus *Mallada* (=*Anisochrysa*), which is the largest of the Chrysopidae with at least 122 described species worldwide (Brooks and Barnard, 1990). *M. basalis* is widely distributed throughout the southwest Pacific

region (Adams, 1959); it is a native species in Taiwan and a natural enemy of insect pests. The larvae of *M. basalis* are generalist predators and has been recorded feeding on aphid, whitefly, mealy bug, thrips, psyllids, mites and eggs and early larval stages of lepidopteran caterpillars (Cheng and Chen, 1996; Wu and Lin, 1998; Lu and Wang, 2005; Sirimachan et al., 2005; Lu and Wang, 2006). Earlier studies have shown that the survival and development of green lacewing (*Chrysoperla carnea*) were negatively affected when they were fed with lepidopteran larvae (*Ostrinia nubilalis* or *Spodoptera littoralis*) reared on an artificial diet containing Cry1Ab, or when the predator was fed directly with the toxin (Hilbeck et al., 1998 a&b, 1999; Dutton et al., 2002). However, subsequent studies have shown that Cry1Ab poses a negligible risk for this predator (Romeis et al., 2004).

Parasitoids play a vital role in the biological control of diamondback moth (*Plutella xylostella*), one of the cosmopolitan crucifer insect pests causing severe yield losses worldwide. *P. xylostella* can be brought under reasonable control by a guild of introduced parasitoids including *Cotesia plutellae*, *Diadegma semiclausum* and *Diadromus collaris* present in South- and Southeast Asia as well as East and Southern Africa (Talekar and Shelton, 2003; Gichini et al., 2008; Kfir, 2004). *B. thuringiensis* biopesticides have widely been used against *P. xylostella* and it is the first insect reported to have evolved resistance to a Cry toxin (Tabashnik et al., 1990; Shelton et al., 1993). Because of the intensive applications, the parasitoids of *P. xylostella* might also have been exposed to *B. thuringiensis* biopesticides or to the Cry toxins when the host insect feeds on the Bt transgenic plants. Direct exposure of *C. plutellae* to *B. thuringiensis* or indirect exposure through *P. xylostella* feeding on Bt oilseed rape did not adversely affect the parasitoid (Chilcutt and Tabashnik, 1999; Schuler et al., 2004). Exposure of *D. insulare*, a close relative of *D. semiclausum*, to Bt plants expressing Cry 1C did not harm the parasitoids (Chen et al., 2008).

The objective of this study was to determine the effects of sub-lethal concentrations of Cry 1B and Cry 1C toxins on the predator, *M. basalis* and the parasitoids, *D. semiclausum* and *C. plutellae*. Furthermore, to have a proper comparison, a commercial formulation of *B. thuringiensis* (Xentari®) containing Cry 1C as a major toxin was also chosen.

MATERIALS AND METHODS

Insects

A *P. xylostella* colony is maintained at the insectary of AVRDC - The World Vegetable Center. The larvae had no contacts with Bt toxins in rearing facilities and they were reared on 10- to 11-week-old potted cabbage plants at 27±1°C, and 70±10% RH, L14:D10 h. The *D. semiclausum* and *C. plutellae* were reared on *P. xylostella*. Eggs of the generalist predator, green lacewing (*M. basalis*) were purchased from a commercial insectary.

B. thuringiensis Cry toxins and formulation

Cry1Ba2 and Cry1Ca4 purified toxins were obtained from R. Akhurst, Commonwealth Scientific and Industrial Research Organization (CSIRO), Canberra, Australia. They were produced in *Escherichia coli* and trypsin activated, and used in all the experiments. Xentari® (Valent BioSciences Corporation, USA), a formulation based on *B. thuringiensis* subsp. *aizawai* was also included in the experiment involving parasitoids.

Sub-lethal doses for Cry toxins and *B. thuringiensis* formulation

Bioassays were performed with *P. xylostella* larvae using leaf dip bioassay IRAC method no. 7. The lethal concentrations causing 50% mortality (LC_{50}), its 95% fiducial limits (FL) and the slope value of probit line were obtained (Shelton et al., 2009). Two other sub-lethal doses *viz.,* LC_{25} and LC_{75} were also estimated. The LC_{25}, LC_{50} and LC_{75} values for Cry1Ba2 are 0.35, 0.65 and 1.22 ppm, respectively, and the values are 0.38, 1.57 and 6.50 ppm, respectively for Cry1Ca4; they are 47, 130 and 368 ppm, respectively for Xentari®.

Effects of Cry toxins on *M. basalis*

M. basalis eggs were purchased from Lacewing Natural Agriculture Co Ltd., Hsinchu, Taiwan. When the eggs hatched into first instar larvae, they were used for the experiments. All experiments were carried out in a controlled room as described above for *P. xylostella* rearing. Early second instar larvae of *P. xylostella* were fed for 8 h on cabbage leaf discs treated with distilled water or LC_{25}, LC_{50} and LC_{75} doses of Cry1Ba2, or Cry1Ca4. In the second method, early second instar larvae of *P. xylostella* were directly dipped in distilled water or LC_{25}, LC_{50} and LC_{75} doses of Cry1Ba2, or Cry1Ca4. The lacewing larvae were fed five Cry toxins fed or treated *P. xylostella* larvae daily, and every other day this diet was supplemented with *Ephestia kuehniella* eggs. Thus, there were four treatments with five replications per treatment in each experiment. Ten lacewing larvae were used for each replication (*i.e.*, 50 larvae per treatment). Green lacewing larvae were checked daily to evaluate the following parameters: larval survival, pupation, pupal survival adult emergence, and development time. When the lacewing adults emerged, pairs of one male and one female were put together in cages, and the number of eggs per female was recorded. The eggs were put aside to record the larval hatching. Data were transformed using *arc-sine* (for the variables defined as percentages) or square root (for the variables defined as numbers). The analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) for mean comparisons were used to determine if there were significant differences among the four treatments.

Effects of Cry toxins and Bt formulation on *D. semiclausum* and *C. plutellae*

Fresh larvae of *P. xylostella* and fresh adults of parasitoids were collected from AVRDC's insect rearing facilities. Early second instar larvae of *P. xylostella* were fed for 24 h on cabbage leaf discs treated with distilled water or LC_{25}, LC_{50} and LC_{75} doses of Cry1Ba2, Cry1Ca4 or Xentari®. Twenty-five *P. xylostella* larvae per replication from each dose were placed on cabbage leaves in acrylic jars (15 cm in diameter, 30 cm in length) and then exposed to two pairs of mated adults of *D. semiclausum* for 24 h, after which adult parasitoids were discarded and parasitized *P. xylostella* larvae were maintained at 27±1°C until *D. semiclausum* and/or *P. xylostella* adults emerged from them. There were four treatments with five replications per treatment in each experiment. Twenty-five *P. xylostella* larvae were used for each replication (*i.e.*, 125 larvae per treatment). Larval survival, pupation and adult emergence of *P. xylostella*, pupation, pupal weight and adult emergence of the parasitoid were recorded. When the parasitoid adults emerged, the same experiment was repeated with the F_1 generation of *D. semiclausum*. Data were transformed using *arc-sine* for the variables defined as percentages. The analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) for mean comparisons were used to determine if there were significant differences among the four treatments. The same experiment was also conducted using *C. plutellae*.

RESULTS AND DISCUSSION

Effects of Cry toxins on *M. basalis*

The Cry1Ba2 toxin did not have any significant negative effects on the larval survival (F=1.09 and P=0.37), pupal survival (F=1.28 and P=0.30), adult emergence (F=2.38 and P=0.94), development time (F=2.07 and P=0.12), fecundity (F=0.88 and P=0.46) and egg hatchability (F=2.49 and P=0.08) of green lacewing larvae reared on *P. xylostella* larvae fed with sub-lethal doses of Cry1Ba2 (LC_{25}, LC_{50} and LC_{75}) compared to the check (Table 1). The same conclusion was drawn for all the parameters studied, including larval survival (F=0.29 and P=0.83), pupal survival (F=0.34 and P=0.79), adult emergence (F=0.52 and P=0.67), development time (F=1.27 and P=0.30), fecundity (F=0.75 and P=0.53) and egg hatchability (F=0.51 and P=0.68) of green lacewing larvae reared on *P. xylostella* larvae treated with sub-lethal doses of Cry1Ba2 (LC_{25}, LC_{50} and LC_{75}) compared to the check (Table 1).

Although Cry1Ca4 toxin did not have any significant negative effects on the larval survival (F=0.44 and P=0.73), development time (F=2.24 and P=0.10), fecundity (F=1.87 and P=0.15) and egg hatchability (F=0.45 and P=0.72) of green lacewing larvae reared on *P. xylostella* larvae fed with sub-lethal doses of Cry1Ca4 (LC_{25}, LC_{50} and LC_{75}) compared to the check (Table 2), there were significant differences among the treatments in pupal survival (F=7.34 and P=0.0006) and adult emergence (F=3.96 and P=0.02). The pupal mortality was highest at LC_{75} dose (25.67%), followed by LC_{25} dose (13.81%) and it was lowest in the check (6.22%). Value for LC_{50} dose was intermediate (10.65%) and similar to either of the extremes. The pupal mortality cannot be correlated with the Cry1Ca4 toxin, since the mortality at higher dose (LC_{50}) is lesser than a lower dose (LC_{25}). However, there were no significant differences among the treatments for all the parameters studied, including larval survival (F=0.32 and P=0.81), pupal survival (F=0.18 and P=0.91), adult emergence (F=0.06 and P=0.98), development time (F=0.94 and P=0.43), fecundity (F=0.10 and P=0.96) and egg hatchability (F=0.61 and P=0.61) of green lacewing larvae reared on *P. xylostella* larvae treated with sub-lethal doses of Cry1Ca4 (LC_{25}, LC_{50} and LC_{75}) compared to the check (Table 2).

There were no earlier studies documenting the effects of either Cry1Ba2 or Cry1Ca4 on *M. basalis* or *C. carnea*, which is one of the widely used green lacewings. However, increased larval mortality of *C. carnea* was reported when they were fed on *O. nubilalis* or *S. littoralis* larvae which were reared on a Bt transgenic corn expressing *cry1Ab* (Hilbeck et al., 1998a). Similar results were also obtained when the predator larvae were fed on host larvae that were reared on artificial diet containing Cry1Ab toxin (Hilbeck et al., 1998b; Hilbeck et al., 1999). In another experiment with Dipel® sprayed maize plants, Dutton et al. (2003) used *S. littoralis* and spider mite (*Tetranychus urticae*) as hosts for rearing the green lacewing. Although *S. littoralis* is susceptible to the Bt toxins, *T. urticae* is unaffected by the Bt toxins. Because the adverse effects were found in lacewing larvae fed on *S. littoralis*, but not on *T. urticae*, they concluded that the detrimental effects were due to the inferior quality of the herbivore prey and not because of the direct toxic effects of the Bt toxin. This was subsequently confirmed by Romeis et al. (2004) who found that *C. carnea* larvae were not sensitive to Cry1Ab and the earlier reported negative effects of Bt-maize were prey-quality mediated rather than direct toxic effects. Rodrigo-Simon et al. (2006) also confirmed that the Cry1Ac, Cry1Ab, or Cry2Ab toxins, even at concentrations higher than those expected in real-life situations, did not have a detrimental effect on the green lacewing when they were ingested either directly or through the prey. Thus, our results are also consistent with earlier studies that showed no direct effects of Cry toxins on green lacewings. In addition, the life history parameters of *M. basalis* in our study also followed the normal pattern. For instance, Pao (2000) had documented that *M. basalis* needed 28.1 d to complete its life cycle, and on average, each female adult could lay about 13.7 eggs/d. In the current study, it took about 22.69-26.35 d to complete its life-cycle, and the fecundity varied from 12.84 to 29.38 eggs/d. Hence, it can be concluded that the Cry1Ba2 and Cry1Ca4 toxins do not have any adverse effects on *M. basalis* at their sub-lethal doses.

Effects of Cry toxins and Bt formulation on *C. plutellae*

The Cry1Ba2 toxin did not have any significant negative effects on the pupation (F=2.47 and P=0.08), pupal weight (F=1.10 and P=0.36) and adult emergence (F=0.01 and P=1.00) of *C. plutellae* that fed and developed in *P. xylostella* larvae fed with sub-lethal doses of Cry1Ba2 (LC_{25}, LC_{50} and LC_{75}) compared to the check (Table 3). In addition, there was also no difference in host (*P. xylostella*) pupation (F=0.29 and P=0.83) among the treatments. When the F_1 progenies of *C. plutellae* emerging from these treatments were used to parasitize the *P. xylostella* larvae fed on diets treated with LC_{25}, LC_{50} and LC_{75} doses of Cry1Ba2 toxin and the check, similar results were obtained for pupation (F=2.87 and P=0.07) and adult emergence (F=0.72 and P=0.55) of *C. plutellae*, as well as the host (*P. xylostella*) pupation (F=1.03 and P=0.41). Although there were variations recorded in pupal weight (F=3.59 and P=0.04) of *C. plutellae* among the treatments, it could not be attributed due to Cry1Ba2 toxin, since the highest pupal weight (1.60 mg) was recorded for parasitoids developed in *P. xylostella* larvae fed on LC_{50} dose, followed by the LC_{25} dose (1.50 mg). The pupal weight of *C. plutellae* in untreated check (1.30 mg) is on par with the highest dose (LC_{75}) of Cry1Ba2 toxin that recorded 1.20 mg.

The Cry1Ca4 toxin also did not affect the parasitism of *P. xylostella* and the development of *C. plutellae*, because there were no significant differences in pupation (F=1.68 and P=0.19), pupal weight (F=2.25 and P=0.10) and adult emergence (F=0.69 and P=0.57) of *C. plutellae* that fed and developed in *P. xylostella* larvae fed with sub-lethal doses of Cry1Ca4 (LC_{25}, LC_{50} and LC_{75}) compared to the check (Table 4). The host (*P. xylostella*) pupation also did not differ significantly (F=1.02 and P=0.41) among the treatments. Similar results were obtained when the F_1 progenies of *C. plutellae* emerging from these sub-lethal treatments were used to parasitize the *P. xylostella* larvae fed on diets treated with LC_{25}, LC_{50} and LC_{75} doses of Cry1Ca4 toxin and the check.

The host (*P. xylostella*) pupation differed significantly (F=4.78 and P=0.005) among the treatments, when they were fed on diets treated with no or sub-lethal doses (LC_{25}, LC_{50} and LC_{75}) of Xentari®. The lowest pupation (12.67%) was recorded in LC_{75} dose, whereas the highest pupation (26.83%) was recorded in LC_{50} dose. Values for all other treatments were intermediate and similar to either of the extremes (Table 5). The highest *C. plutellae* pupation was recorded in untreated check (54.50%), which was on par with the LC_{25} dose recording 48.82% (F=2.85 and P=0.05). It was significantly lowest (37.45%) in LC_{75} dose, whereas it was intermediate and similar to either of the extremes for the LC_{50} dose. Although the pupation rate differed, there were no significant differences in pupal weight (F=0.88 and P=0.46) and adult emergence (F=1.99 and P=0.13) of *C. plutellae* that fed and developed in *P. xylostella* larvae fed with sub-lethal doses (LC_{25}, LC_{50} and LC_{75}) of Xentari® compared to the check. However, no such variations in host (*P. xylostella*) or parasitoid (*C. plutellae*) pupation were obtained when the F_1 progenies of *C. plutellae* emerging from these sub-lethal treatments were used to parasitize the *P. xylostella* larvae fed on diets treated with LC_{25}, LC_{50} and LC_{75} doses of Xentari® and the check.

Effects of Cry toxins and Bt formulation on *D. semiclausum*

Neither Cry1Ba2 toxin nor Cry1Ca4 toxin did have any significant adverse effect on the host (*P. xylostella*) pupation (F=0.22; P=0.89 and F=2.20; P=0.10, respectively) or the pupation (F=1.46; P=0.24 and F=1.02; P=0.39, respectively), pupal weight (F=0.13; P=0.94 and F=0.16; P=0.92, respectively) and adult emergence (F=1.17; P=0.33 and F=0.26; P=0.86, respectively) of *D. semiclausum* that fed and developed in *P. xylostella* larvae fed on diets with sub-lethal doses (LC_{25}, LC_{50} and LC_{75}) of Cry1Ba2 and Cry1Ca4 compared to the check (Tables 6 and 7). Similar results were also obtained when the F_1 progenies of *D. semiclausum* emerging from these sub-lethal treatments were used to parasitize the *P. xylostella* larvae fed on diets treated with LC_{25}, LC_{50} and LC_{75} doses of Cry1Ba2 and Cry1Ca4 toxins and the check.

Although Xentari® did not affect the host (*P. xylostella*) pupation significantly (F=0.32 and P=0.81) among the treatments when it was fed on diets treated with no or sub-lethal doses (LC_{25}, LC_{50} and LC_{75}), it reduced the *D. semiclausum* pupation significantly (Table 8). The lowest pupation (46.24%) was recorded in LC_{75} dose, whereas the highest pupation (74.56%) was recorded in the untreated check, and it was on par with the LC_{25} and LC_{50} doses recording 67.44% and 64.69%, respectively (F=6.62 and P=0.0007). Although the pupation rate differed, there were no significant differences in pupal weight (F=0.43 and P=0.73) and adult emergence (F=2.64 and P=0.06) of *D. semiclausum* that fed and developed in *P. xylostella* larvae fed with sub-lethal doses (LC_{25}, LC_{50} and LC_{75}) of Xentari® compared to the check. However, no such variation in *D. semiclausum* pupation was recorded when the F_1 progenies of *D. semiclausum* emerging from these sub-lethal treatments were used to parasitize the *P. xylostella* larvae fed on diets treated with LC_{25}, LC_{50} and LC_{75} doses of Xentari® and the check.

To our knowledge, the effects of Cry1Ba2 and Cry1Ca4 toxins on the larval parasitoids (*D. semiclausum* and *C. plutellae*) of *P. xylostella* were not reported earlier. In the current study, we did not record any major adverse effects of either Cry1Ba2 or Cry1Ca4 on *C. plutellae*. Basically, *C. plutellae* adults did not discriminate the host (*P. xylostella*) larvae either exposed or unexposed to the Cry toxins, which was reflected in the host and parasitoid pupation rates. This fact was also supported by earlier findings by Chilcutt and Tabashnik (1999) who reported that the mean number of ovipositions by *C. plutellae* in Bt treated *P. xylostella* larvae was not significantly different from untreated larvae. It also holds

good for *D. semiclausum* in the current study. In addition to oviposition, the Bt toxins also did not affect the parasitoids' growth and development. Schuler et al. (2004) found that when *C. plutellae* parasitized Cry1Ac-resistant *P. xylostella* that had fed on Cry1Ac-expressing oilseed rape, there was no effect of Bt plants on percentage parasitism, time to emergence from hosts, time to adult emergence and percentage adult emergence from cocoons, which suggested that Cry1Ac was not toxic to *C. plutellae*. Similarly, Cry1C resistant *P. xylostella* was fed on Bt transgenic broccoli plants producing a high level of Cry1C (1.09–1.12 mg/g fresh leaf tissue) and then become parasitized by *Diadegma insulare*, an important endoparasitoid of *P. xylostella* in North America, the parasitoid was exposed to a biologically active form of the Cy1C protein while in the host, but the parasitism and parasitoid adult emergence were not harmed by such exposure (Chen et al., 2008). Thus, the results from the current study provide some evidence of the lack of hazard to *C. plutellae* and *D. semiclausum* by Cry1Ba2 and Cry1Ca4.

The effects of several formulations of *B. thuringiensis* subsp. *aizawai* (Bt*a*) or *B. thuringiensis* subsp. *kurstakii* (Bt*k*) on these natural enemies were reported. Kao and Tzeng (1992) classified Bt*k* based formulations as slightly harmful to *C. plutellae*. In a more recent study by Haseeb et al. (2004), Crymax® and Xentari® had no effects on *C. plutellae* adults, whereas Mattch® and Dipel® caused about 5-10% mortality. There was a decline in the pupation rate of *C. plutellae* and *D. semiclausum*, when they developed in the host fed on diets containing sub-lethal doses of Xentari® in the current study. Grbin (1997) reported that a sub-lethal dose of Bt not only slowed down the development of *P. xylostella* but also resulted in slower development of *C. plutellae* larvae inside the surviving hosts. The parasitoid *Cotesia marginiventris* developed slower in soybean looper (*Pseudoplusia includens*) fed on Bt cotton expressing Cry1Ac toxin because of the slower larval development of *P. includens* (Baur and Boethel, 2003). Hence, it is possible that the development of the parasitoids could be affected when they develop inside a host fed on *B. thuringiensis* formulations that usually contain more than one Cry toxin. Although we observed the decline in the pupation rate in F_0 generation, it did not affect the F_1 generation. Similar variations across the generations have already been reported. The larval development time of *Cotesia flavipes* in the host insect (sugarcane borer, *Diatraea saccharalis*) was longer in one of two generations tested when the host's artificial diet was incorporated with sugarcane leaves expressing snowdrop lectin (*Galanthus nivalis* agglutinin, GNA) (Setamou et al., 2002).

CONCLUSION

The Cry1Ba2 and Cry1Ca4 toxins had sometimes marginal effects on the pupal survival or adult emergence of the generalist predator, green lacewing (*M. basalis*). Although Cry1Ba2 and Cry1Ca4 toxins did not adversely affect the parasitoids (*C. plutellae* and *D. semiclausum*), Xentari® reduced the pupation rate. However, these adverse effects on the predator and the parasitoids were not consistent across the experiments or generations. Hence, the variations in the results of the studies outlined above may be related to the degree to which the host insect (*P. xylostella*) is sub-lethally affected by the insecticidal Cry toxins and the degree to which the insecticidal Cry toxins penetrates the host's tissues, as explained by Schuler et al. (2004). It could also be correlated to the inferior host-quality mediated effects on the natural enemies (Chen et al., 2008). Thus, it could be concluded that the selected Cry1Ba2 and Cry1Ca4 toxins are relatively safer to the natural enemies of *P. xylostella* such as *M. basalis*, *C. plutellae* and *D. semiclausum*.

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Table 1. Effect of Cry 1B toxin fed and treated diamondback moth (DBM) on growth and development of green lacewing, *Mallada basalis*

Dose (ppm)	Cry 1B toxin fed DBM on *M. basalis*						Cry 1B toxin treated DBM on *M. basalis*					
	Larval mortality (%)*	Pupal mortality (%)*	Eclosion (%)*	Dev. time (d)**	Fecundity (no.)**	Egg hatchability (%)*	Larval mortality (%)*	Pupal mortality (%)*	Eclosion (%)*	Dev. time (d)**	Fecundity (no.)**	Egg hatchability (%)*
Check	3.00 a (5.76)	15.78 a (19.31)	82.00 a (69.16)	25.78 a (5.13)	14.03 a (3.59)	78.80 a (63.65)	6.00 a (9.05)	19.19 a (24.31)	75.00 a (62.19)	24.80 a (5.03)	21.36 a (4.27)	75.75 a (61.63)
0.35 (LC_{25})	6.00 a (10.20)	19.17 a (24.24)	76.00 a (61.29)	25.61 a (5.11)	12.84 a (3.35)	66.00 a (54.08)	7.00 a (9.65)	22.47 a (26.44)	72.00 a (60.22)	25.90 a (5.14)	28.31 a (5.14)	83.81 a (67.39)
0.65 (LC_{50})	6.00 a (10.20)	27.33 a (29.81)	68.00 a (55.95)	26.35 a (5.18)	16.10 a (3.97)	82.44 a (65.84)	5.00 a (6.03)	20.44 a (26.37)	76.00 a (61.37)	25.87 a (5.13)	19.22 a (3.98)	71.03 a (57.54)
1.22 (LC_{75})	10.00 a (14.45)	23.81 a (27.42)	69.00 a (58.06)	26.01 a (5.15)	17.00 a (4.12)	86.73 a (70.01)	10.00 a (11.73)	23.75 a (28.57)	67.00 a (55.69)	25.45 a (5.09)	21.58 a (4.52)	77.31 a (62.47)
F value	1.09	1.28	2.38	2.07	0.88	2.49	0.29	0.34	0.52	1.27	0.75	0.51
P value	0.37	0.30	0.94	0.12	0.46	0.08	0.83	0.79	0.67	0.30	0.53	0.68

* Figures in parentheses are arc-sine transformed values

** Figures in parentheses are square-root transformed values

Values followed by same letter(s) are not significantly different at p=0.05

Table 2. Effect of Cry 1C toxin fed and treated diamondback moth (DBM) on growth and development of green lacewing, *Mallada basalis*

Dose (ppm)	Cry 1C toxin fed DBM on *M. basalis*						Cry 1C toxin treated DBM on *M. basalis*					
	Larval mortality (%)*	Pupal mortality (%)*	Eclosion (%)*	Dev. time (d)**	Fecundity (no.)**	Egg hatchability (%)*	Larval mortality (%)*	Pupal mortality (%)*	Eclosion (%)*	Dev. time (d)**	Fecundity (no.)**	Egg hatchability (%)*
Check	5.00 a (9.38)	6.22 c (10.40)	88.00 a (70.94)	22.69 a (4.81)	19.21 a (4.27)	82.02 a (65.56)	3.00 a (5.76)	14.45 a (19.70)	82.00 a (66.57)	25.11 a (5.06)	21.50 a (4.52)	83.79 a (66.81)
0.38 (LC_{25})	7.00 a (11.00)	13.81 b (19.52)	80.00 ab (64.88)	23.09 a (4.86)	17.73 a (4.04)	76.29 a (60.63)	6.00 a (10.20)	16.28 a (22.27)	79.00 a (64.57)	25.67 a (5.12)	22.73 a (4.50)	77.46 a (62.07)
1.57 (LC_{50})	7.00 a (9.86)	10.65 bc (18.02)	83.00 a (66.23)	23.29 a (4.88)	29.38 a (5.30)	83.50 a (66.20)	7.00 a (9.65)	14.78 a (19.06)	80.00 a (66.56)	25.48 a (5.10)	24.82 a (4.83)	88.55 a (71.24)
6.50 (LC_{75})	3.00 a (5.76)	25.67 a (29.84)	72.00 b (58.54)	23.23 a (4.87)	21.63 a (4.63)	80.51 a (65.13)	5.00 a (8.39)	14.36 a (18.49)	80.00 a (66.74)	25.44 a (5.09)	25.72 a (4.75)	77.49 a (62.70)
F value	0.44	7.34	3.96	2.24	1.87	0.45	0.32	0.18	0.06	0.94	0.10	0.61
P value	0.73	0.0006	0.02	0.10	0.15	0.72	0.81	0.91	0.98	0.43	0.96	0.61
LSD	-	8.47	7.37	-	-	-	-	-	-	-	-	-

* Figures in parentheses are arc-sine transformed values

** Figures in parentheses are square-root transformed values

Values followed by same letter(s) are not significantly different at p=0.05

Table 3. Effect of Cry 1Ba2 on *Cotesia plutellae*

Dose (ppm)	F_0				F_1			
	DBM Pupation (%)	*C. plutellae* Pupation (%)	*C. plutellae* Pupal weight (mg)	*C. plutellae* Adult emergence (%)	DBM Pupation (%)	*C. plutellae* Pupation (%)	*C. plutellae* Pupal weight (mg)	*C. plutellae* Adult emergence (%)
Check	15.01 (21.92)	61.79 (52.03)	1.70	91.97 (76.34)	10.40 (18.42)	78.40 (62.76)	1.30^{b}	97.82 (84.40)
0.35	14.27 (20.47)	68.88 (56.35)	1.70	90.11 (77.17)	4.80 (9.78)	51.20 (45.70)	1.50^{ab}	87.50 (76.66)
0.65	14.41 (20.25)	58.75 (47.26)	1.70	89.85 (77.50)	8.80 (12.88)	57.60 (50.77)	1.60^{a}	96.10 (81.11)
1.22	19.27 (24.00)	53.12 (46.78)	1.60	89.79 (77.28)	4.00 (9.01)	69.60 (56.73)	1.20^{b}	98.82 (81.11)
F value	0.29	2.47	1.10	0.01	1.03	2.87	3.59	0.72
P value	0.83	0.08	0.36	1.00	0.41	0.07	0.04	0.55
LSD	-	-	-	-	-	-	0.0003	-

Figures in parentheses are arc-sine transformed values
Values followed by same letter(s) in a column are not significantly different at p=0.05

Table 4. Effect of Cry 1Ca4 on *Cotesia plutellae*

Dose (ppm)	F_0				F_1			
	DBM Pupation (%)	*C. plutellae* Pupation (%)	*C. plutellae* Pupal weight (mg)	*C. plutellae* Adult emergence (%)	DBM Pupation (%)	*C. plutellae* Pupation (%)	*C. plutellae* Pupal weight (mg)	*C. plutellae* Adult emergence (%)
Check	13.18 (19.83)	70.65 (58.14)	1.90	94.80 (80.61)	7.20 (13.66)	53.60 (47.15)	1.40	98.34 (86.39)
0.38	19.32 (25.01)	76.01 (61.86)	1.90	96.26 (81.31)	3.20 (6.56)	65.60 (54.38)	1.80	97.80 (84.39)
1.57	9.74 (15.94)	83.31 (66.92)	1.80	97.93 (84.55)	5.60 (13.49)	56.00 (48.57)	1.80	91.48 (76.77)
6.50	8.68 (15.00)	70.96 (57.93)	1.80	93.19 (78.85)	5.60 (10.76)	72.80 (59.6)	1.70	98.76 (86.85)
F value	1.02	1.68	2.25	0.69	0.86	2.34	1.47	1.47
P value	0.41	0.19	0.1	0.57	0.48	0.11	0.26	0.26

Figures in parentheses are arc-sine transformed values

Table 5. Effect of Xentari® on *Cotesia plutellae*

Dose (ppm)	**F_0**				**F_1**			
	DBM Pupation (%)	*C. plutellae* Pupation (%)	*C. plutellae* Pupal weight (mg)	*C. plutellae* Adult emergence (%)	DBM Pupation (%)	*C. plutellae* Pupation (%)	*C. plutellae* Pupal weight (mg)	*C. plutellae* Adult emergence (%)
Check	21.37 (27.07)ab	54.50 (47.66) a	1.70	93.70 (80.14)	15.23 (22.12)	59.35 (50.54)	1.90	92.21 (77.47)
47	16.35 (22.87)bc	48.82 (44.37) a	1.70	95.89 (82.53)	17.87 (22.99)	63.51 (53.56)	1.80	93.91 (80.27)
130	26.83 (30.46)a	46.77 (42.77) ab	1.80	93.04 (79.5)	13.11 (18.93)	72.01 (58.64)	1.90	93.14 (79.44)
368	12.67 (19.58)c	37.45 (36.16) b	1.60	84.00 (70.26)	19.1 (25.16)	64.59 (54.16)	1.80	93.15 (77.89)
F value	4.78	2.85	0.88	1.99	1.11	1.83	0.52	0.21
P value	0.0050	0.05	0.46	0.13	0.35	0.15	0.57	0.89
LSD	0.17	8.12						

Figures in parentheses are arc-sine transformed values
Values followed by same letter(s) in a column are not significantly different at p=0.05

Table 6. Effect of Cry 1Ba2 on *Diadegma semiclausum*

Dose (ppm)	F_0				F_1			
	DBM pupation (%)	*D. semiclausum* pupation (%)	*D. semiclausum* pupal weight (mg)	*D. semiclausum* adult emergence (%)	DBM pupation (%)	*D. semiclausum* pupation (%)	*D. semiclausum* pupal weight (mg)	*D. semiclausum* adult emergence (%)
Check	7.73 (14.12)	74.85 (61.30)	4.70	93.08 (78.62)	2.28 (5.64)	65.52 (54.45)	4.10	89.68 (73.82)
0.35	6.29 (11.27)	68.43 (57.03)	4.70	87.68 (72.21)	13.64 (20.24)	67.30 (55.71)	4.50	81.04 (65.41)
0.65	6.73 (11.86)	71.81 (58.52)	4.70	92.19 (78.20)	6.58 (9.67)	72.60 (58.46)	4.40	83.66 (67.37)
1.22	8.79 (12.80)	61.43 (53.13)	4.80	93.40 (78.59)	7.34 (15.51)	57.52 (49.39)	4.40	84.46 (70.14)
F value	0.22	1.46	0.13	1.17	2.43	0.89	2.97	0.48
P value	0.89	0.24	0.94	0.33	0.10	0.47	0.06	0.70

Figures in parentheses are arc-sine transformed values

Table 7. Effect of Cry 1Ca4 on *Diadegma semiclausum*

Dose (ppm)	F_0				F_1			
	DBM pupation (%)	*D. semiclausum* pupation (%)	*D. semiclausum* pupal weight (mg)	*D. semiclausum* adult emergence (%)	DBM pupation (%)	*D. semiclausum* pupation (%)	*D. semiclausum* pupal weight (mg)	*D. semiclausum* adult emergence (%)
Check	13.39 (20.39)	64.24 (56.28)	4.60	82.78 (68.23)	3.82 (7.37)	71.68 (57.99)	4.10	74.88 (60.60)
0.38	10.73 (17.81)	68.15 (56.28)	4.70	86.22 (70.52)	5.18 (8.04)	63.36 (52.92)	4.10	92.00 (75.57)
1.57	9.55 (13.82)	69.45 (57.22)	4.70	82.14 (67.38)	3.02 (6.58)	66.60 (54.89)	4.40	82.30 (67.88)
6.50	11.77 (19.4)	62.05 (52.25)	4.70	81.75 (67.47)	1.42 (3.36)	62.04 (52.06)	4.40	87.22 (71.46)
F value	2.20	1.02	0.16	0.26	0.24	1.13	1.95	1.66
P value	0.10	0.39	0.92	0.86	0.87	0.37	0.16	0.22

Figures in parentheses are arc-sine transformed values

Table 8. Effect of Xentari® on *Diadegma semiclausum*

Dose (ppm)	**F_0**				**F_1**			
	DBM Pupation (%)	*D. semiclausum* Pupation (%)	*D. semiclausum* Pupal weight (mg)	*D. semiclausum* Adult emergence (%)	DBM Pupation (%)	*D. semiclausum* Pupation (%)	*D. semiclausum* Pupal weight (mg)	*D. semiclausum* Adult emergence (%)
Check	7.16 (11.70)	74.56 (60.70) a	5.00	83.83 (67.68)	7.08 (15.33)	69.12 (57.19)	4.30	68.96 (57.33)
47	10.34 (12.75)	67.44 (56.21) a	4.70	69.71 (58.50)	12.14 (20.25)	63.62 (53.01)	4.80	76.50 (62.26)
130	4.98 (9.68)	64.69 (53.98) a	4.50	85.13 (69.31)	7.14 (13.28)	64.24 (54.18)	4.70	57.00 (49.54)
368	5.80 (8.79)	46.24 (42.47) b	4.50	72.35 (60.35)	10.84 (18.48)	66.92 (55.05)	4.80	87.40 (71.42)
F value	0.32	6.62	0.43	2.64	1.39	0.16	2.06	2.80
P value	0.81	0.0007	0.73	0.06	0.28	0.92	0.15	0.07
LSD		8.55						

Figures in parentheses are arc-sine transformed values
Values followed by same letter(s) in a column are not significantly different at p=0.05

SESSION 5

Insecticides and insecticide resistance

Update on DBM diamide resistance from the Philippines: causal factors and findings

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ABSTRACT
Flubendiamide (Fenos®) and Chlorantraniliprole (Prevathon®) insecticides, representing the IRAC Mode of Action Group 28, were registered in the Philippines in 2006 and 2007 respectively. Fenos® and Prevathon® are novel diamide products thus providing growers excellent rotation partners to manage insecticide resistance development in vegetables. These products quickly became very popular among growers since they were very effective against diamondback moth and other lepidopteran larvae. Baseline monitoring for insect susceptibility to chlorantraniliprole was conducted by DuPont from 2006 to 2008 at locations in Benguet and Laguna, showing high sensitivity to the diagnostic dose rates of 1 and 5 ppm. However, beginning in 2009, field representatives from other locations were reporting that efficacy against diamondback moth appeared to diminish. Throughout 2010 field performance failures were reported from Cebu. Subsequent susceptibility monitoring from multiple locations in Cebu showed low mortality rates for both chlorantraniliprole and flubendiamide at the highest diagnostic dose rate of 5ppm. Cross resistance by diamondback to both diamide products appears evident. Additional monitoring at locations in Negros Oriental also showed reduced susceptibility at 1 and 5 ppm compared to earlier assays conducted from the northern islands. In response, the Philippines IRAC Diamide Working Group conducted a survey among Cebu growers to better understand the causal factors leading to the development of diamondback moth resistance to the diamides. Over-dependency on Fenos® and Prevathon®, continuous plantings, over and under dosing, and lack of a sound rotation program are some of the key factors leading to insect resistance. Further details identifying causal factors will be reported.

Keywords
Insecticide Resistance, Diamides, DBM, Philippines, Flubendiamide, Chlorantraniliprole

INTRODUCTION
Even before the introduction of the newest group of insecticides, there have been reports of Diamondback Moth (Plutella xylostella) developing tolerance to the different products that have been used in the field for some time. Since concerned farmers didn't make any prior adjustments but saw the reduction in the efficacy of the crop protection products, they initially thought the companies producing them lessened the amount of active ingredients in the formulation. Some thought that such observed reduction in susceptibility only happened to insecticides that were chemically synthesized. But use of microbial disruptors such as Bacillus thuringiensis subsp. kurstaki were also later reported to need the introduction of the subsp. aizawai to ensure they remain effective longer in the field. The DBM has coped with previously introduced control options in the field. Despite this, many hoped the introduction of a novel group of crop protection materials against lepidopterous pests would be good enough to protect the cruciferous crops for a long

time. Within four years of the first introduction of the diamide family of compounds, reports that DBM in another cabbage-growing location in Cebu, Philippines have shown reduced susceptibility to the diamide products currently on the market. This paper aims to present the efforts of CropLife Philippines and their member companies to verify and determine factors that may influence the development of reduced susceptibility of the DBM to the diamide class of crop protection products.

Crop protection alternatives

Belonging to the IRAC Mode of Action Group 28, the diamide insecticides, Flubendiamide (Fenos®) from Bayer Crop Science and Chlorantraniliprole (Prevathon®) from DuPont Far East, Inc., are novel diamide products which when launched as a means of controlling DBM in 2006 and 2007, respectively, were quickly accepted by cabbage growers. Voliam Flexi®, a premix of Chlorantraniliprole and Thiamethoxam, was also introduced later by Syngenta. Packaged to become excellent rotation partners to the current products, their proven efficacy against the resistant DBM and other lepidopterous insects in crucifers made them very popular and widely used.

Reports of DBM with low susceptibility

More than 2 years after being introduced, Flubendiamide was still providing good control against DBM in the highlands of Benguet. This is according to the results of susceptibility tests conducted by Bayer from 2006 to 2009. Based on studies comparing susceptibility data from 2006-2008 by DuPont, the same performance was observed for Chlorantraniliprole in that area. However, in September of 2009, some field representatives covering Cebu area received reports of reduced control of DBM using the diamide pesticides. With no previous data on the DBM in the Cebu area, studies were conducted to establish the level of susceptibility in 2010. The data generated indicated that the DBM in that area needed a higher amount of toxicant to control the target pest.

Survey of farm practices

Responding to the reports of observed reduction in susceptibility to the diamide treatments, the companies distributing the products formed the Diamide Working Group under the Stewardship Committee of CropLife Philippines, Inc. Composed of the combined field representatives of the three companies, a quick survey to learn about possible factors that influenced the observed tolerance levels of the DBM in the Cebu area was launched. Data was gathered from 100 cabbage farmers on August 20-24, 2010.

Table 1 shows the summary of the survey conducted. It was observed that diamide compounds were the dominant products used throughout the cabbage growing season.

Based on the observations, it was recommended that a more comprehensive study program be carried out in order to arrive at more conclusive results and sound recommendations for the use of Diamide insecticides in conjunction with crop protection products with other modes of action. Other insect resistance management strategies will also be incorporated in the implementation**.**

Table 1. Summary of survey results conducted in Sudlon, Cebu, Philippines on August 20-24, 2010

USAGE PARAMETER	OBSERVED FARMER PRACTICE
Incidence of use	*High incidence of diamide use* • *Use of diamide compounds is very pronounced* • *Flubendiamide and Chlorantraniliprole are the more popular products* • *Use of multiple diamide brands suggest rotation only within the diamide family*
Frequency of spray	*12 sprays in a season; around 70% or higher use of diamide products* *Share of Flubendiamide slightly ahead of Chlorantraniliprole, in terms of % share of sprays*
Product Rotation	*Rotation with compounds from other chemical classes/modes of action is generally not practiced* • *Very few practice diamide rotation with compounds from other chemical modes of action* • *Brand rotation is more pronounced than compound/mode of action rotation*
Product Mixing	• *Incidence of tank-mixing with other insecticide compounds, with different modes of action, is very low* • *For those who tank-mix, there was mention of fungicides as well as insecticides in the tank-mix*
Dose rate applied	*Observed overdosing* • *Average dose for diamide compounds was higher than the recommended rates.* • *Those who practiced overdosing perceived lower efficacy of the product against DBM*

Other influencing factors

Based on susceptibility studies conducted by Bayer Crop Science and DuPont Far East, Inc., the DBM in the highland areas of Benguet are more susceptible compared to the DBM from the cabbage-growing areas of Cebu.

Aside from the differences in the farmers' cultural practices in the production of cabbage, the climatic conditions of the two locations also differ considerably.

The highlands of Benguet located 1500 – 3900 ft. above sea level has a mean temperature range of 18.5 – 23 degrees centigrade. The midlands of Cebu, on the other hand, has production areas within the elevation of 100 – 2845 ft above sea level with warmer mean temperatures of 25-28 degrees centigrade.

Studies have shown that temperature and relative humidity play a big role in the life cycle duration of the DBM. They revealed that under warmer temperatures the total life cycle of the DBM tends to be shorter.(Wai et al. 2008, Mau and Kessing 2007).

In the typical Filipino farm where planting is not synchronized, there are related crops as well as alternate hosts growing throughout the year. This cropping system ensures the continuous development of insects. It is observed that different generations usually overlap with all insect stages present in the field at the same time (Wai et al. 2008).

On the part of the crop protection industry, past efforts to manage resistance have been done as independent entities not as coordinated groups. As a result, these were not sustained widely and long enough to be adopted by many farmers to make an impact. The government sector also had limited impact whenever they launched programs.

What needs to be done

The different stakeholders wanted to do something. But with uncoordinated efforts, resistance development will continue at an unprecedented pace. There is a need for everyone to get involved in a coordinated way. Companies should develop green chemical alternatives that can be used as additional options to integrated pest management and sustainable crop production. The government researchers, the academe and non-government entities should work together to disseminate the package of technology that will be implemented with discipline by farmers; with the help of local government units. Making this big program work will be a daunting task. But unless something is done now, food production will continue to be an expensive and difficult endeavor for all concerned.

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Update on DBM diamide resistance from Thailand: situation and causal factors

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ABSTRACT
Flubendiamide (Takumi® 20WDG) insecticide, representing the IRAC Mode of Action Group 28, was registered in Thailand in May, 2007. Takumi® is a novel diamide product that offered growers excellent control of diamondback moth (DBM) and other lepidopteran larvae in a crucifer market where few other insecticides were adequately effective. Baseline monitoring conducted earlier by DuPont indicated that chlorantraniliprole, also a diamide insecticide, showed lower levels of susceptibility to diamondback larvae compared to that found in other countries. DBM larvae in Thailand are historically notorious for their speed of developing resistance to new products. It is speculated that metabolic mechanisms play a major role in the development of insect resistance. Field observations in 2009 from the Bang BuaThong area indicated that Takumi® was not providing adequate control resulting in growers changing their spraying practices to compensate. Field and lab testing showed that DBM larvae had indeed developed resistance to flubendiamide with cross resistance to chlorantraniliprole. Resistance factors for flubendiamide and chlorantraniliprole in Sai Noi population, Nonthaburi province, were 66.3 and 35.4 respectively in 2010. Some of the key factors leading to insect resistance were over-dependency on a single mode of action, continuous plantings of crucifers, minimal crop rotation to non-cruciferous crops, underdosing, irrigation practices that led to excessive product wash-off and exposure to sublethal levels, and lack of insecticide resistance management (IRM) strategies. Further details identifying additional causal factors, including grower pest control behavior and practices, will be reported.

Keywords
Insecticide Resistance, DBM, Flubendiamide, Thailand, Chlorantraniliprole

INTRODUCTION
Insecticide resistance in the diamondback moth has been a great concern for all farmers who grow vegetables in Thailand. This is the most important reason why insecticides with a new mode of action have been desired. To preserve the efficacy of such insecticides as long as possible, all causal factors of resistance occurring locally should be investigated. This knowledge could be applied to prevent severe damage caused by the resistant diamondback moth in the future.

Diamondback moth, *Plutella xylostella* (L.), is one of the most destructive insect pests of cruciferous vegetables in Thailand and around the world. The control cost for it in the early 1990s was average US$1 billion annually (Talekar and Shelton 1993). Generally, the control of this pest in Thailand depends solely on the use of synthetic insecticides. Due to rapid generation time, high reproductivity, and particularly the exposure to high selection pressure in the field, these attributes have resulted in *P. xylostella* evolving resistance to various types of insecticides. Obviously, it has been recorded that the diamondback moth rapidly can develop resistance to many classes of insecticides (Liu et al. 1997). Nevertheless, *P. xylostella* has proved to have the potential ability to evolve resistance to any insecticides sooner or later after extensive field use (Talekar and Shelton 1993). To date, *P. xylostella* has developed resistance to 81 insecticides (APRD 2009) and has become one of the most difficult pests to control in cruciferous vegetables in Thailand. Recently, Thai farmers have reported that flubendiamide showed reduced efficacy for controlling diamondback larvae in some areas. Baseline monitoring conducted earlier by DuPont indicated that chlorantraniliprole also showed lower levels of susceptibility to diamondback larvae compared to that found in other countries.

Flubendiamide (Takumi® 20WDG) is a novel insecticide, representing the IRAC Mode of Action Group 28 (ryanodine receptor modulator) within the IRAC (Insecticide Resistance Action Committee) mode of action classification scheme. Flubendiamide is the first member of phthalic acid diamides, and is active against a broad range of lepidopteran insects (Tohnishi et al. 2005; Nauen 2006). Takumi® was registered in Thailand in May, 2007. This insecticide offered growers very effective control of diamondback moth and other

lepidopteran larvae in crucifers. The excellent efficacy of flubendiamide against a resistant *P. xylostella* strain was also reported (Tohnishi et al. 2005). Additionally, flubendiamde shows an excellent biological and ecological profile (Hall 2007; Hirooka et al. 2007). Thus, flubendiamide was hoped to be an excellent tool for controlling lepidopteran insects as a part of insecticide resistance management and integrated pest management (IPM) programs. However, due to the injudicious use of this insecticide by most of Thai farmers, reduced efficacy in regard to insecticide resistance has been claimed.

Chlorantraniliprole (Prevathon® 5%SC) is also a novel insecticide from a new class of chemistry, the IRAC Mode of Action Group 28. Chlorantraniliprole is the first member of anthranilic diamides, and is potent within the insect order Lepidoptera (Temple et al. 2009). Chlorantranilprole is relatively harmless to beneficial arthropods, and has not been found to exhibit cross resistance with existing insecticides (Lahm et al. 2009). These favorable characteristics provide an additional management tool to control *P. xylostella* and to make it a good tool for IPM and IRM strategies. However, this insecticide belongs to the same mode of action group 28 as flubendiamide, and the possibility of cross resistance between these two insecticides should be considered.

Introducing IRM strategies to farmers is necessary in order to mitigate the resistance of the diamondback moth to the diamide insecticides, the most frequently used insecticides for the control of this pest. The monitoring insecticide resistance status is important for forecasting the failure of insecticide control in combination to IPM in the future. This paper therefore reports the change in susceptibility to flubendiamide and chlorantraniliprole in some diamondback moth populations collected from the most important vegetable growing areas in Central Thailand from 2008 to 2011. The information obtained will be used for planning insecticide resistance management strategy for the diamondback moth in Thailand.

MATERIALS AND METHODS

Insect cultures

Field populations were collected from the important vegetable growing areas from central Thailand - Sai Noi district, Nonthaburi province; Tha Muang district, Kanchanaburi province; and Tub Berg district, Phetchabun province from 2008 to 2011. About 300 larvae or pupae were collected from each sampling site. Cultures of all populations were established in laboratory at the Pest Management Group, Office of Plant Protection Research and Development, Department of Agriculture, Thailand. Adults that emerged from the field populations were provided with 10% honey solution in cotton wool and mass-mated at random in cages. Adults were allowed to lay eggs on crinkled sheets of aluminum foil. The emerged larvae were fed young cabbage leaves. Third instar of F1 larvae were used for bioassays. All populations were maintained at 27 $\pm$ 1^{o}C.

Insecticides used

Commercial formulations of diamide insecticides, flubendiamide (Takumi 20%WDG; TJC Chemical Company Ltd., Bangkok, Thailand) and chlorantraniliprole (Prevathon 5% SC; DuPont (Thailand) Limited, Bangkok, Thailand), were used in this study. The insecticides were prepared with reversed osmosis water containing 0.025% spreading agent (Tension T-7, Sotus International Company, Ltd., Nonthaburi, Thailand).

Leaf dip bioassay

The flubendiamide and chlorantraniliprole formulations were diluted to generate serial dilutions with reversed osmosis water containing 0.025% Tension T-7. The leaf-dip method was used for bioassays (Ninsin et al. 2000). Cabbage, *Brassica oleracea* L., leaves measuring 5 cm x 5 cm were cut and dipped in different concentrations of insecticides for 10 s and then allowed to air dry at room temperature. Control leaves were dipped in water containing 0.025 % Tension T-7 only. Each leaf was then placed in individual 100-cm^3 plastic cup containing a single filter paper. Ten third instar larvae were released into each cup. An average of five concentrations of each insecticide was used. Each concentration and control was replicated four times. Larvae were then kept at 27 $\pm$ 1^{o}C and allowed to feed on the treated leaf for 48 h. The mortality was assessed after 48 h. Larvae that did not respond to pencil-tip prodding were considered to be dead.

Statistical analysis

The mortality data were analyzed by probit analysis to estimate the median lethal concentrations (LC50) and the 95% fiducial limits (FL) (Finney 1971). Mortality was corrected using Abbott's formula (Abbott 1925). Differences in susceptibility between populations were considered to be significant when the 95% FL of LC50 values did not overlap. The bioassay results indicated that the Tub Berg population tested in 2009 was the most susceptible to flubendiamide and chlorantraniliprole and thus it was used in calculating the Resistance factors (RFs). Then, RF values were calculated by dividing the LC50 of a field population by that of the most susceptible Tub Berg population.

RESULTS AND DISCUSSION

The diamondback moth population in Thailand firstly showed evidence of resistance to flubendiamide and chlorantraniliprole between years 2008 to 2010, around three years after the registration of flubendiamide in Thailand. The Sai Noi population from Nonthaburi province, the nearest vegetable growing area around Bangkok, showed higher resistance (RF=66.3) to flubendiamide than that of chlorantraniliprole (RF=35.4) when using Tub Berg population, the most susceptible field population, for comparison (Table 1). Furthermore, Tha Muang population from Kanchanaburi province initially showed some tolerance to flubendiamide (RF=1.5) (Table 1).

In year 2011, Sai Noi and Tha Muang populations have still showed evidences of flubendiamide and chlorantraniliprole resistance. This year, Sai Noi population also showed higher resistance (RF=407.2) to flubendiamide than that of chlorantraniliprole (RF=152.7) (Table 2). Interestingly, Tha Muang population showed very high increase of resistance to flubendiamide (RF=4,817.4) and high resistance to chlorantraniliprole (RF=87.7) (Table 2).

Thai farmers are generally concerned about resistance of insect pests to insecticides. The resistance in DBM directly impacts quality and yield of crucifers. After its registration in Thailand, Takumi® was a diamide product that offered growers excellent control of DBM and other lepidopteran larvae in a crucifer market where few other insecticides were adequately effective (Andaloro et al. 2011). In recent years, the resistance of diamide insecticides in DBM larvae from Thailand has been increasing dramatically. The resistance factor of Tha Muang population, Kanchanaburi province, and Sai Noi population, Nonthaburi province, to flubendiamide have increased from 1.5 to 4,817 and from 66.3 to 407, respectively (Tables 1 and 2). Likewise, the resistance factor of Sai Noi population to chlorantraniliprole has increased from 35.4 to 152. These data explained the rapid increase of field doses used by farmers and proved complaints of putative resistance to diamide insecticides in Thailand.

Table 1. Susceptibility of diamondback moth larvae in Thailand to flubendiamide and chlorantraniliprole from 2008 to 2010

Insecticide	Population[1]	n[2]	Slope ± SE	LC_{50} (95%FL) [mg/liter]	RF[3]
Flubendiamide	Sai Noi	320	0.463 ± 0.081	10.6 (3.84-22.8)	66.3
	Tha Muang	300	0.451 ± 0.058	0.246 (0.113-0.593)	1.5
	Tub Berg	300	1.254 ± 0.129	0.160 (0.0366-0.811)	-
Chlorantraniliprole	Sai Noi	320	0.709 ± 0.091	7.97 (4.09-13.7)	35.4
	Tub Berg	200	1.593 ± 0.237	0.225 (0.0535-0.587)	-

[1] Tha Muang, Tub Berg, and Sai Noi population was tested in 2008, 2009, and 2010 respectively.

[2] Number of larvae used in bioassay, including control.

[3] Resistance factor = LC_{50} of a population / LC_{50} of the Tub Berg population, the most susceptible field population tested.

Table 2. Susceptibility of diamondback moth larvae in Thailand to flubendiamide and chlorantraniliprole in Feb-Mar 2011

Insecticide	Population	n[1]	Slope ± SE	LC_{50} (95%FL) [mg/liter]	RF[2]
Flubendiamide	Sai Noi	151	0.695 ± 0.235	65.1 (2.71-157)	407.2
	Tha Muang	180	0.619 ± 0.135	771 (123-26,336)	4,817.4
	Tub Berg[3]	300	1.254 ± 0.129	0.160 (0.0366-0.811)	-
Chlorantraniliprole	Sai Noi	156	0.986 ± 0.238	34.4 (12.1-60.6)	152.7
	Tha Muang	240	1.200 ± 0.160	19.7 (7.32-92.4)	87.7
	Tub Berg[3]	200	1.593 ± 0.237	0.225 (0.0535-0.587)	-

[1] Number of larvae used in bioassay, including control.

[2] Resistance factor = LC_{50} of a population / LC_{50} of the Tub Berg population, the most susceptible field population tested.

[3] Tub Berg population was tested in 2009.

In early 2008, the diamide products could be rotated with other lepidopteran products without concern of cross resistance. However, recent evidence in Asian countries where diamide resistance to *Plutella xylostella* occurred indicated that cross resistance was highly likely among the Group 28 products (Andaloro et al. 2011). Field observations in 2009 from Bang Bua Thong, Nonthaburi province, indicated that Takumi® was not providing adequate control. The field-recommended dose from the bottle label of flubendiamide to DBM is only 60 mg/liter, whereas in 2011, Tha Muang population showed higher LC50 of 771 mg/liter and Sai Noi population also showed higher LC50 of 65 mg/liter (Table 2). In regard to many cases of cross resistance in *P. xylostella,* resistance risk to chlorantraniliprole still exists. Therefore, the severity of resistance of diamondback moth to diamide insecticides might be higher in the future in most vegetable growing areas of Thailand where over-dependency and excessive use of this

insecticide have already been extensively exploited. As a result, the useful life of diamide insecticide in Thailand may not extend.

In the near future, the insecticide resistance situation could be a major limiting factor of vegetable production in Thailand. Situations in the Philippines tend to be the same as that in Thailand. In 2009, Philippines field representatives were reporting that efficacy against diamondback moth appeared to diminish. Throughout 2010 field performance failures were reported from Cebu province. Subsequent susceptibility monitoring from multiple locations in Cebu showed low mortality rates for both chlorantraniliprole and flubendiamide at the highest diagnostic dose rate of 5 ppm. Cross resistance by diamondback to both diamide products appears evident. Additional monitoring at locations in Negros Oriental also showed reduced susceptibility at 1 and 5 ppm compared to earlier assays conducted from the northern islands (Edralin et al. 2011). On the other hand, the reduced susceptibility among 16 field populations of diamondback moth from China was still low, with LC50 values varying from 0.221 to 1.104 mg/liter (Wang et al. 2010).

Many factors were responsible for insecticide resistance in DBM from Thailand. Such factors included frequent spraying, over-dependency on a single mode of action, continuous plantings of crucifers, minimal crop rotation to non-cruciferous crops, underdosing, irrigation practices that led to excessive product wash-off and exposure to sublethal levels and lack of insecticide resistance management strategies. Actually, Thai farmers used flubendiamide more than 4 to 5 times per crop in tank mixes with other insecticides to control the target pest and other pests at the same time to reduce labor costs for spraying. Unfortunately, the use of insecticide mixtures by farmers could increase severity of resistance in DBM, if the resistance gene already existed in high frequency (Georghiou 1983). These farmers' practices may explain the rapid occurrence of diamide resistance in DBM populations from Thailand.

The diamide insecticide resistance in DBM from Thailand has been increasing considerably. Therefore, the urgent action should be performed to retard the severity of resistance. However, resistance management strategies should begin at the very start of the lifespan of a new pesticide and not in response to a perceived problem (Jutsum et al. 1998). To control *P. xylostella* effectively and extend the useful life of diamide insecticides, judicious use of this compound including limiting spray times and windows, appropriate doses, and correct application techniques should be considered essential from its earliest use in the field (Wang et al. 2010). In this regard, the insecticides that show no cross resistance to the diamide insecticide in DBM populations from Thailand should be identified. The management of spraying by using window strategy and effective insecticide partners for rotation should be extensively utilized. IPM practices including crop rotation or crop-free periods should be maximized to decrease selection pressure of diamide and other insecticides.

CONCLUSION

Diamide resistance in DBM populations from Thailand has been increasing dramatically. Therefore, the urgent action should be performed to reduce the resistant DBM cases. In this regard, the insecticides that show no cross resistance to the diamide insecticide in DBM populations from Thailand should be identified. Spraying by using window strategy and effective insecticide partners for rotation as well as IPM practice should be extensively utilized.

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Some Australian populations of diamondback moth, *Plutella xylostella* (L.) show reduced susceptibility to fipronil

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ABSTRACT
Fipronil has been used for control of diamondback moth (DBM), *Plutella xylostella* (L.), on *Brassica* vegetables in Australia since its registration as Regent® 200 SC in 1997. It was the first of several new chemistries registered after pyrethroid resistance was experienced with DBM in the 1980s. Its use declined as other products became available, some of which have less severe effects on natural enemies of DBM and are more appropriate for integrated pest management (IPM).

As part of a National Insecticide Resistance Management Strategy, baseline susceptibility of eight Australian DBM populations to fipronil was assessed using leaf dip bioassays at 22°C in 1996/97, prior to first field use of the insecticide. Five populations were equivalent to the standard Waite laboratory population with respect to fipronil susceptibility. However, a population from South Australia and two populations from Queensland showed elevated tolerance to fipronil compared with the standard. After registration, testing of DBM continued and populations were screened with both fipronil and endosulfan, given concern about cross-resistance with cyclodienes. One year after baseline estimates, four DBM populations from Queensland showed the highest levels of tolerance to both fipronil and endosulfan compared with levels in populations from other states. Tolerance ratios to fipronil of three of the four Queensland DBM populations were higher than those of the baseline Queensland populations. Tolerance of DBM from 5 southern states had not changed significantly from baseline estimates. Although Queensland DBM populations showed tolerance to fipronil, field performance of the insecticide should not have been jeopardised because LC90 values were still low compared with the registered use rate. Because of grower concern about poor performance of fipronil in Queensland in winter, effect of temperature on susceptibility of DBM was assessed at 14°C, 18°C, 22°C and 28°C. There was a significant decline in susceptibility at lower temperatures, which would have contributed to the observed field efficacy problems.

Keywords
fipronil, endosulfan, cross-resistance, insecticide resistance management, temperature coefficient of toxicity

INTRODUCTION
Resistance to pyrethroids in populations of *Plutella xylostella* was widespread throughout all vegetable *Brassica* areas of Australia by the mid-1990s (Deuter 1989; Baker and Kovaliski 1999; Endersby et al. 2008; Eziah et al. 2009). Resistance was subsequently found in populations of *P. xylostella* from broad-acre canola crops throughout the southern cropping areas of Australia (Endersby et al. 2008; Furlong et al. 2008). Nerve insensitivity by mutations in the *para* sodium channel gene has been found to be a common pyrethroid resistance mechanism in *P. xylostella* in Australia (Endersby et al. 2011).

The Australian agrochemical industry has promoted an Insecticide Resistance Management Strategy to rationalise the use of new insecticides that were becoming available to Australian growers since the late 1990s. In 1997, AVCARE's Insecticide Resistance Action Committee (AIRAC), in consultation with researchers, devised a two-window insecticide resistance management strategy for *P. xylostella*. Essentially, new insecticides were to be used in one of two production windows each year to avoid a mosaic of insecticide groups being used in a production region (Ashby et al. 1998; Roush et al. 1998). In early 1998, only fipronil had been registered for control of DBM. As other insecticides became registered for use against *P. xylostella* on vegetable brassicas, they were allocated to one of the two windows. The actual dates of the use windows varied between regions. For example, the current strategy for *Brassica* growers in the Lockyer Valley in Queensland is as follows:

> **Window 1** (1 February - 15 June): fipronil (first registered September 1997), emamectin benzoate (first registered February 2000), chlorantraniliprole (first registered September 2008) and flubendiamide (first registered April 2009).
>
> **Window 2** (16 June - 31 October): chlorfenapyr (first registered November 1998), spinosad (first registered September 1999), indoxacarb (first registered January 2001)

The vegetable *Brassica* growers in the Lockyer Valley, Queensland were the first Australian growers to use fipronil widely to control *P. xylostella* (IRM window: February–June 1998). However, they were disappointed with the field performance of the insecticide, in terms of speed of kill and level of mortality. We undertook further bioassays of field populations to assess susceptibility levels of *P. xylostella* in the region and to investigate the role of temperature on the efficacy of fipronil.

Fipronil acts by blocking chloride channels associated with γ-amino-butyric acid (GABA) receptors in the insect central nervous system (Grant et al. 1998; Bloomquist 2001; Nakao et al. 2010, 2011). It also inhibits glutamate-activated chloride channels (GluCls) (Zhao et al. 2003; Narahashi et al. 2010). Because of the action of fipronil on the GABA-gated chloride channel, cross-resistance has been reported in some insects between fipronil and cyclodiene insecticides such as dieldrin (Scott and Wen 1997; Gao et al. 2007). Resistance to fipronil in *P. xylostella* has now been reported in field populations in China (Zhou et al. 2011), India (Mohan and Gujar 2003), Indonesia (Budiarto and Setiawati 2007), Malaysia (Sayyed and Wright 2004; Idris et al. 2004) and Taiwan (Kao and Cheng 2001). Li et al. (2006) have suggested that *A302S* mutation in the *PxRdl* gene is partially associated with fipronil resistance in *P. xylostella.*

Insecticide toxicity can be greatly influenced by temperature (Smith et al. 1994; Scott 1995; Musser and Shelton 2005). The increasing metabolic activity of insects as temperature increases may either enhance the toxic effects of the insecticide or increase insect ability to detoxify or tolerate insecticides (Scott 1995). There had been no information published about the influence of temperature on the toxicity of fipronil to *P. xylostella* at the time of the study. Kumar and Chapman (1983) had demonstrated a marked increase in toxicity of permethrin and fenvalerate to *P. xylostella* at 15°C compared to 25°C.

This paper provides data on the susceptibility of field populations of *P. xylostella* in Australia before field use (baseline) and after use by growers, investigates possible interactions of fipronil susceptibility with endosulfan resistance and assesses the influence of temperature on fipronil efficacy.

MATERIALS AND METHODS

Bioassays with fipronil and endosulfan

Field collected *P. xylostella* larvae were reared on cabbage seedling leaves (cv. Green Coronet) in the laboratory at 25°C (16h: 8h, L: D). A susceptible laboratory population of *P. xylostella* was obtained from the University of Adelaide, Department of Crop Protection Waite Campus, SA, in 1994 and was reared in the laboratory at Knoxfield continuously since then. At the request of Rhône-Poulenc Ag. Co. North Carolina, USA, the standard leaf dip bioassay method, originally outlined by Tabashnik and Cushing (1987), was modified. Cabbage (*Brassica oleracea* var. *capitata* cv. Green Coronet) leaf discs of 4.5 cm diameter were dipped for 5 s in distilled water solutions of formulated fipronil (Regent 200 SC formulation) 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 Gelman 50 x 9 mm plastic Petri dishes. Five third instar *P. xylostella* were added to each disc and allowed to feed at 22°C. Mortality was assessed at 24, 48 and 72 h. Dead larvae were those which did not move when touched with a paintbrush. Probit analysis (POLO-Plus, LeOra Software) was used to estimate LC_{50} and slope for each population. A similar method was used for testing endosulfan except that four replicates of ten larvae per disc were set up for each concentration and larvae were allowed to feed at 28ºC for 48 h. Lethal dose ratios were calculated using the susceptible Waite population as the reference (the susceptible Waite population was included in every series of bioassays of field populations to act as an internal control).

Before fipronil was registered in Australia (September 1997) for use against *P. xylostella* on vegetable *Brassica* crops, baseline bioassays were conducted on eight populations of *P. xylostella* collected from vegetable *Brassica* crops in Queensland (two populations), South Australia (two populations), New South Wales, Victoria, Tasmania and Western Australia (Figure 1). Bioassays were conducted between September 1996 to August 1997.

In 1998, further fipronil bioassays were conducted on 11 populations of *P. xylostella* (Queensland x 4; Victoria x 3; New South Wales; South Australia; Tasmania; Western Australia). All of these populations were also assessed for endosulfan susceptibility. As part of a much larger insecticide resistance monitoring program, a further 23 populations of *P. xylostella* collected from vegetable *Brassica* crops were tested for fipronil susceptibility (seven populations in 2000; six populations in 2001; six populations in 2002; four populations in 2003).

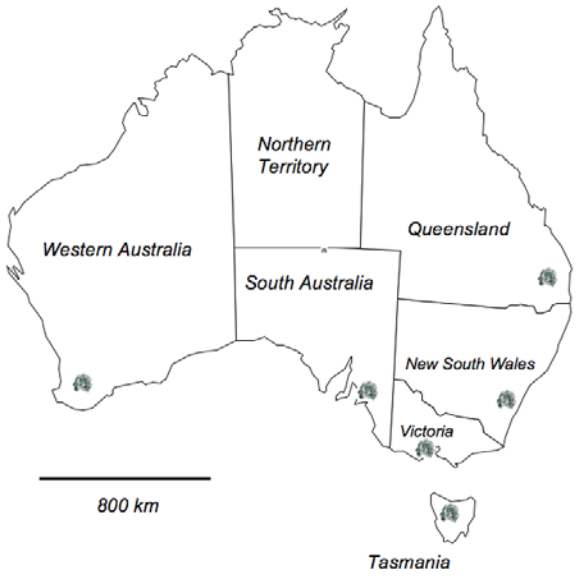

Figure 1. Location of collection sites for *P. xylostella* populations from vegetable *Brassica* growing areas in Australia

Effect of temperature on fipronil susceptibility

In 1998, the susceptibility of a Lockyer Valley, Queensland population of *P. xylostella* and the susceptible Waite population to 9 ppm fipronil was assessed at three temperatures (14ºC, 20ºC and 28ºC) using the fipronil leaf dip bioassay method (eight replicates (n=40) for the

Lockyer Valley population; four replicates (n=20) for the susceptible Waite population). A physiological time scale, using a lower development threshold for *P. xylostella* of 7.4ºC (Liu et al. 2002) was used when comparing the results between temperatures. Detailed fipronil bioassays (0, 0.09, 0.3, 0.5, 0.7, 1.0, 3.0 ppm) were conducted at four temperatures (10ºC, 18ºC, 22ºC and 28ºC) for the susceptible Waite population to examine intrinsic effect of temperature on the toxicity of fipronil. The effect of fluctuating temperature on fipronil susceptibility was examined at 14ºC using the Waite population. Groups of larvae were exposed to leaf discs treated with 1 ppm or untreated. Larvae were then held in temperature cabinets either at a constant 14ºC or in a cycling temperature (mean 14ºC) which ranged from 20ºC to 9ºC (moving down 1ºC each hour to 9ºC then moving up 1ºC each hour to 20ºC). Mortality was assessed every 24 hours.

RESULTS

Bioassays with fipronil and endosulfan

Baseline (pre-registration) susceptibility to fipronil

Five populations of *P. xylostella* (from New South Wales, South Australia, Victoria, Tasmania and Western Australia) were as susceptible to fipronil as the unsprayed Waite laboratory population (Table 1), but two populations from Queensland and a second population from South Australia were significantly less susceptible than the Waite population.

Fipronil and endosulfan susceptibility

Testing with fipronil in 1998 and 1999 also showed that the four populations of *P. xylostella* from the Lockyer Valley, Queensland, were much less susceptible to fipronil than the susceptible control population (Table 2). Six of the seven populations tested from the southern states were also significantly less susceptible than the susceptible control population, but were significantly more susceptible than the Queensland populations. Ten of the 11 populations were also significantly less susceptible to endosulfan than the susceptible control population (Table 3). Again the four Queensland populations were significantly less susceptible than six of the seven populations from the southern states. There was a strong Spearman's rank correlation (r=0.80) between fipronil and endosulfan lethal dose ratios (Figure 2).

Further testing between 2000 and 2003 (23 field populations) (Table 4) showed that 12 populations were significantly less susceptible to fipronil than the susceptible laboratory population. Again four of the five Queensland populations tested were the least susceptible. By 2002 and 2003, the level of resistance observed had decreased, with one population collected in Gatton, Queensland in 2002 being as susceptible as the control population.

Table 1. Slope, LC_{50} (ppm) and lethal dose ratio (LDR_{50}) for fipronil tested at 22˚C for 72 h on eight Australian *P. xylostella* populations (pre-field use) compared with the standard laboratory population (Waite), (n=number of subjects, s.e.=standard error, C.L.=confidence limits)

Population	n	slope ± s.e.	LC_{50} (95% C.L.) ppm	LDR_{50} (95% C.L.)
Forth, Tas.	240	2.18 ± 0.24	0.56 (0.37–0.86)	0.99 (0.71–1.40)
Gatton, Qld	440	1.50 ± 0.13	2.73 (2.08–3.56)	4.84 (3.53–6.65)
Waite (S)	279	2.53 ± 0.28	0.57 (0.24–1.02)	
Lindenow, Vic.	238	1.33 ± 0.22	0.27 (0.09–0.47)	1.05 (0.58–1.92)
Waite (S)	244	4.26 ± 0.72	0.26 (0.20–0.31)	
Mandogalup, WA	242	1.73 ± 0.24	0.56 (0.32–0.82)	0.95 (0.65–1.40)
Waite (S)	244	2.43 ± 0.31	0.58 (0.40–0.79)	
Clarence Town, NSW	241	1.55 ± 0.25	0.32 (0.09–0.55)	1.21 (0.70–2.08)
Waite (S)	244	2.39 ± 0.34	0.26 (0.17–0.36)	
Stanthorpe, Qld	237	1.37 ± 0.16	2.18 (1.45–3.33)	3.23 (2.10–4.96)
Waite (S)	281	1.95 ± 0.39	0.68 (0.34–1.03)	
Mt Barker, SA	239	1.83 ± 0.23	0.86 (0.56–1.22)	2.60 (1.73–3.91)
Waite (S)	284	2.29 ± 0.38	0.33 (0.21–0.43)	
Nairne, SA	242	1.40 ± 0.17	1.85 (1.35–2.53)	1.55 (1.04–2.29)
Waite (S)	279	1.97 ± 0.28	1.20 (0.92–1.67)	

Table 2. Slope, LC_{50} (ppm) and lethal dose ratio (LDR_{50}) for fipronil tested at 22°C for 72 h on eleven Australian *P. xylostella* populations in 1998 compared with the standard laboratory population (Waite), (n=number of subjects, s.e.=standard error, C.L.=confidence limits)

Population	n	slope ± s.e.	LC_{50} (95% C.L.)	LDR_{50} (95% C.L.)
Lockyer Valley, Qld	240	1.76 ± 0.20	7.74 (5.63–10.88)	10.77 (7.45–15.57)
Waite (S)	280	1.57 ± 0.19	0.72 (0.52–1.04)	
Mt Sylvia, Qld	243	1.55 ± 0.18	8.28 (5.98–11.89)	17.41 (11.57–26.22)
Helidon, Qld	241	1.66 ± 0.18	5.51 (3.92–7.77)	11.59 (7.81–17.20)
Waite (S)	201	1.82 ± 0.34	0.48 (0.26–0.67)	
Castlereagh, NSW	280	1.62 ± 0.19	1.10 (0.73–1.62)	1.97 (1.41–2.75)
Waite (S)	278	2.72 ± 0.36	0.56 (0.46–0.68)	
Devonport, Tas.	280	1.02 ± 0.15	0.93 (0.48–1.55)	1.88 (1.18–3.01)
Werribee Dec 98, Vic.	281	1.25 ± 0.17	1.12 (0.65–1.77)	2.27 (1.46–3.55)
Waite (S)	241	2.47 ± 0.34	0.49 (0.37–0.63)	
South Australia	282	2.28 ± 0.28	0.84 (0.62–1.11)	1.38 (1.02–1.86)
Waite (S)	240	2.20 ± 0.27	0.61 (0.5–0.8)	
Western Australia	280	2.57 ± 0.37	0.52 (0.38–0.65)	1.16 (0.88–1.52)
Waite (S)	240	2.38 ± 0.28	0.45 (0.33–0.58)	
Werribee Feb 99, Vic.	280	1.90 ± 0.23	0.66 (0.43–0.96)	1.57 (1.18–2.09)
Waite (S)	240	2.96 ± 0.38	0.4 (0.35–0.50)	
Werribee Mar 99, Vic.	280	1.71 ± 0.20	0.76 (0.48–1.12)	2.00 (1.42–2.84)
Tenthill, Qld	280	1.91 ± 0.18	2.02 (1.62–2.56)	5.33 (3.81–7.43)
Waite (S)	241	1.99 ± 0.26	0.38 (0.25–0.53)	

Table 3. Slope, LC_{50} (ppm) and lethal dose ratio (LDR_{50}) for endosulfan tested at 28°C for 48 h on eleven Australian *P. xylostella* populations in 1998 compared with the standard laboratory population (Waite) (n=number of subjects, s.e.=standard error, C.L.=confidence limits)

Population	n	slope ± s.e.	LC_{50} (95% C.L.)	LDR_{50} (95% C.L.)
Lockyer Valley, Qld	281	1.68 ± 0.20	2508.4 (1646.8–4475.9)	32.00 (20.33–50.38)
Waite (S)	240	1.66 ± 0.20	78.4 (40.1–123.3)	
Mt Sylvia, Qld	279	1.91 ± 0.19	1486.7 (996.1–2201.6)	5.85 (3.93–8.71)
Helidon, Qld	281	2.26 ± 0.23	1452.3 (1070.7–1929.8)	5.72 (3.85–8.49)
Waite (S)	280	1.40 ± 0.18	254.0 (175.9–362.4)	
Werribee Dec 98, Vic.	280	1.83 ± 0.23	443.9 (299.6–604.8)	1.94 (1.37–2.75)
Devonport, Tas.	280	2.16 ± 0.28	442.9 (325.2–574.4)	1.94 (1.41–2.67)
Waite (S)	280	1.94 ± 0.20	228.3 (185.1–283.6)	
Castlereagh, NSW	241	1.99 ± 0.24	520.8 (357.8–717.7)	1.67 (1.22–2.29)
Waite (S)	285	2.17 ± 0.21	289.0 (247.0–397.3)	
South Australia	279	3.71 ± 0.65	343.4 (272.0–409.8)	1.87 (1.42–2.46)
Western Australia	280	2.73 ± 0.43	321.4 (250.4–391.0)	1.75 (1.31–2.33)
Waite (S)	281	2.62 ± 0.28	183.9 (151.4–222.6)	
Werribee Feb 99, Vic.	280	1.63 ± 0.25	248.8 (124.7–372.4)	1.27 (0.83–1.96)
Waite (S)	280	2.27 ± 0.23	195.9 (154.8–249.0)	
Werribee Mar 99, Vic.	280	2.64 ± 0.28	779.7 (642.8–947.5)	3.53 (2.59–4.80)
Tenthill, Qld	280	2.11 ± 0.20	1799.8 (1323.9–2470.0)	8.14 (5.88–11.26)
Waite (S)	280	1.64 ± 0.18	221.1 (161.6–304.8)	

Table 4. Slope, LC_{50} (ppm) and lethal dose ratio (LDR_{50}) for fipronil tested at 22°C for 72 h on 23 Australian *P. xylostella* populations compared with the standard laboratory population (Waite), 2000–2003 (n=number of subjects, s.e.=standard error, C.L.=confidence limits)

Date	Population	n	Slope ± s.e.	LC_{50} (ppm) (95% CL)	LDR_{50} (95% CL)
15-Mar-00	Nairne, SA	280	2.00 ± 0.29	0.54 (0.36–0.73)	1.88 (1.37–2.58)
15-Mar-00	Waite (S)	242	3.04 ± 0.36	0.29 (0.23–0.34)	
21-Mar-00	Manjimup, WA	280	2.90 ± 0.45	0.47 (0.37–0.56)	1.20 (0.92–1.55)
21-Mar-00	Waite (S)	240	2.64 ± 0.33	0.39 (0.32–0.46)	
09-May-00	Werribee South, Vic.	281	1.34 ± 0.19	0.53 (0.32–0.77)	1.18 (0.80–1.76)
09-May-00	Woolnorth, Tas.	280	1.97 ± 0.27	0.61 (0.45–0.79)	1.35 (0.98–1.86)
09-May-00	Waite (S)	240	2.28 ± 0.28	0.45 (0.37–0.55)	
05-Sep-00	Glenore Grove, Qld	280	1.36 ± 0.15	1.74 (1.30–2.37)	2.95 (2.12–4.11)
05-Sep-00	Castlereagh NSW	286	1.54 ± 0.19	0.71 (0.45–1.01)	1.20 (0.84–1.71)
05-Sep-00	Waite (S)	240	2.80 ± 0.35	0.59 (0.49–0.71)	
04-Dec-00	Grantham, Qld	278	1.38 ± 0.15	2.55 (1.76–3.91)	7.37 (5.01–10.83)
04-Dec-00	Waite (S)	240	1.92 ± 0.25	0.35 (0.21–0.49)	
19-Jun-01	St Kilda, SA	281	1.42 ± 0.16	1.08 (0.78–1.47)	1.40 (0.98–2.02)
19-Jun-01	Waite (S)	240	1.95 ± 0.27	0.77 (0.59–1.04)	
15-Jun-01	Albany, WA	281	1.88 ± 0.20	1.34 (1.01–1.78)	1.46 (1.05–2.03)
15-Jun-01	Waite (S)	241	2.10 ± 0.28	0.92 (0.71–1.25)	
27-Mar-01	Werribee South, Vic.	280	2.25 ± 0.26	0.90 (0.64–1.27)	1.24 (0.95–1.64)
27-Mar-01	Waite (S)	241	2.31 ± 0.29	0.72 (0.57–0.93)	
06-Mar-01	Gawler, Tas.	280	2.05 ± 0.25	0.75 (0.53–1.04)	1.59 (1.21–2.09)
06-Mar-01	Waite (S)	240	3.45 ± 0.56	0.47 (0.30–0.62)	
14-Mar-01	Castlereagh, NSW	280	2.63 ± 0.37	0.79 (0.62–0.98	1.18 (0.91–1.54)
14-Mar-01	Waite (S)	240	3.10 ± 0.43	0.67 (0.54–0.83)	
05-Jun-01	Gatton, Qld	280	1.68 ± 0.18	6.41 (4.71–9.37)	11.27 (7.74–16.42)
05-Jun-01	Waite (S)	240	1.87 ± 0.24	0.57 (0.42–0.76)	
30-Jan-02	Devonport, Tas.	280	1.64 ± 0.17	5.52 (4.07–7.99)	2.13 (1.26–3.61)
30-Jan-02	Waite (S)	240	1.80 ± 0.33	2.59 (1.65–6.47)	
06-May-02	Wanneroo, WA	281	1.84 ± 0.19	1.28 (0.97–1.69)	1.42 (1.07–1.90)
06-May-02	Waite (S)	240	2.66 ± 0.34	0.90 (0.74–1.13)	
17-Jun-02	Lindenow, Vic.	280	1.64 ± 0.18	1.07 (0.77–1.47)	1.34 (0.94–1.90)
17-Jun-02	Cowra, NSW	280	1.68 ± 0.18	1.11 (0.85–1.45)	1.39 (0.99–1.93)
17-Jun-02	Waite (S)	240	2.39 ± 0.40	0.80 (0.62–1.02)	
23-Jul-02	Virginia, SA	280	2.11 ± 0.24	0.95 (0.76–1.19)	1.05 (0.80–1.39)
23-Jul-02	Gatton, Qld	280	2.29 ± 0.26	1.04 (0.75–1.45)	1.16 (0.87–1.54)
23-Jul-02	Waite (S)	240	2.66 ± 0.34	0.90 (0.74–1.13)	
11-Mar-03	Wanneroo, WA	280	2.05 ± 0.19	2.38 (1.85–3.15)	2.37 (1.74–3.23)
11-Mar-03	Waite (S)	240	3.00 ± 0.42	1.00 (0.75–1.35)	
21-Jan-03	Wesleyvale, Tas	280	3.46 ± 0.40	1.18 (0.96–1.53)	1.28 (1.02–1.61)
21-Jan-03	Helidon, Qld	280	2.21 ± 0.20	2.08 (1.52–2.92)	2.26 (1.74–2.94)
21-Jan-03	Werribee South, Vic.	280	2.36 ± 0.23	1.56 (1.17–2.17)	1.70 (1.31–2.19)
21-Jan-03	Waite (S)	240	3.40 ± 0.44	0.92 (0.77–1.16)	

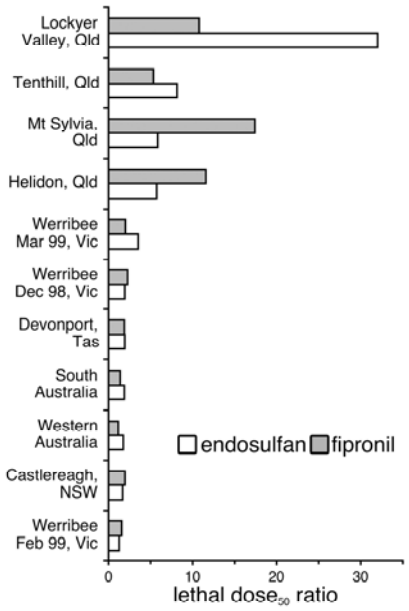

Figure 2. Lethal dose ratios for fipronil and endosulfan for 11 *P. xylostella* populations tested in 1998 and 1999 and compared with the susceptible Waite population.

In all, these tests involved 32 bioassays of the susceptible Waite population with fipronil. The mean LC_{50} for the Waite population tested with fipronil between 1996 and 2003 was 0.67 ± 0.07 ppm.

Effect of temperature on fipronil susceptibility

The reduced susceptibility of the Lockyer Valley population compared to the susceptible Waite population in 1998 (LDR_{50} at 22°C = 10.77) was confirmed in bioassays with 9 ppm fipronil conducted at three temperatures (Figures 3 a, b). In further tests with 1 ppm fipronil, the susceptibility of the Waite population was found to be greatest at 28°C (Figures 4 a, b). This finding was confirmed in the full dose bioassays (Figures 5 a, b). There was no difference in susceptibility to fipronil observed at 14°C between treated *P. xylostella* larvae held either in cycling or constant temperatures (Figure 6). This suggests that the observed mortality was related strongly to the rate of development of larvae and was not influenced by short-term changes in the metabolic rate of the larvae.

DISCUSSION

The well-established low-level cross resistance between cyclodiene insecticides and fipronil (Scott and Wen 1997; Gao et al. 2007; Heckel 2009) was reflected in the lower susceptibility to fipronil and endosulfan observed in populations of *P. xylostella* from Queensland, even before fipronil had been used in the field. Queensland vegetable *Brassica* growers face a wider diversity of lepidopteran pests than do growers

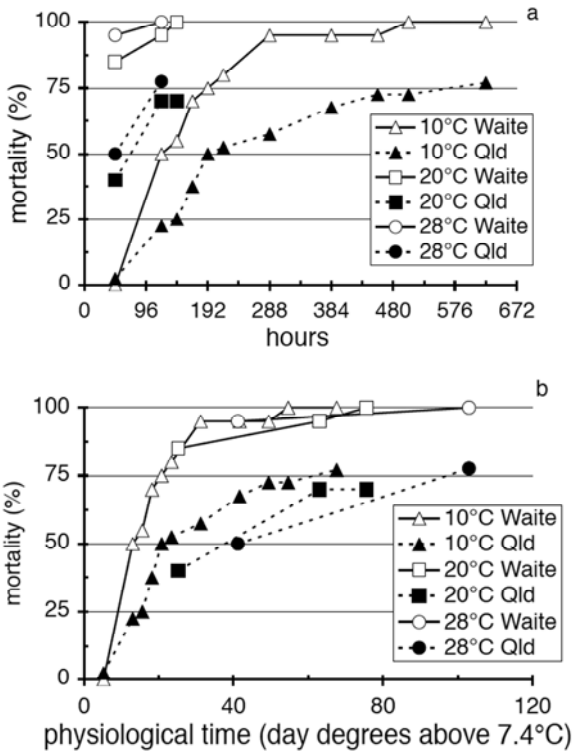

Figure 3. Per cent mortality after exposure to 9 ppm fipronil for a Lockyer Valley, Queensland, population and the susceptible Waite population of *P. xylostella* presented (a) on calendar time scale, (b) on physiological time scale.

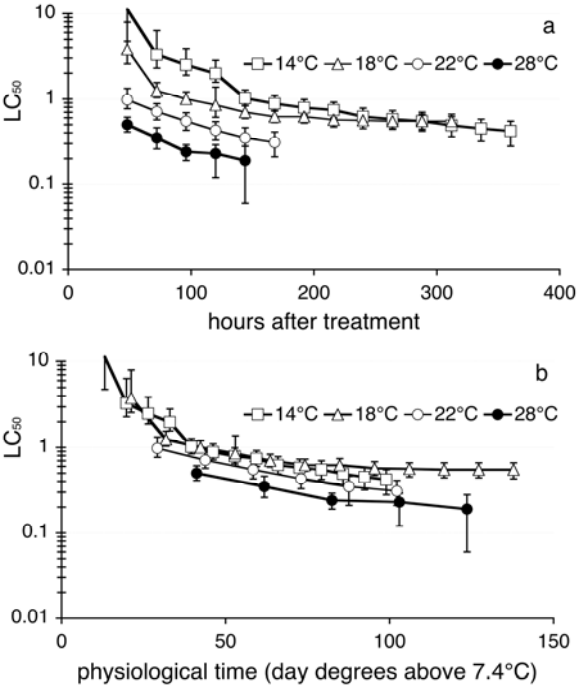

Figure 4. LC_{50} (95% confidence limits) for *P. xylostella* larvae (susceptible Waite population) tested at 4 temperatures presented (a) on calendar time scale, (b) on a physiological time scale (base temperature = 7.4°C)

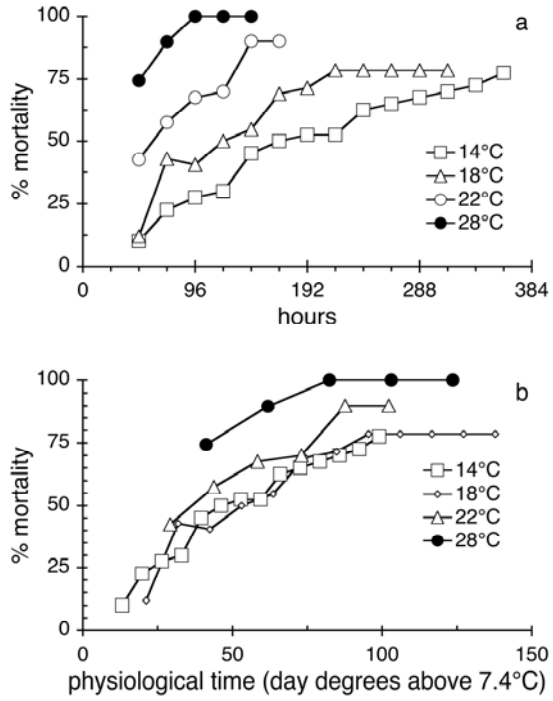

Figure 5. Per cent mortality of *P. xylostella* larvae (susceptible Waite population) exposed to 1 ppm of fipronil at 4 temperatures, presented (a) on calendar time scale, (b) on a physiological time scale (base temperature = 7.4˚C).

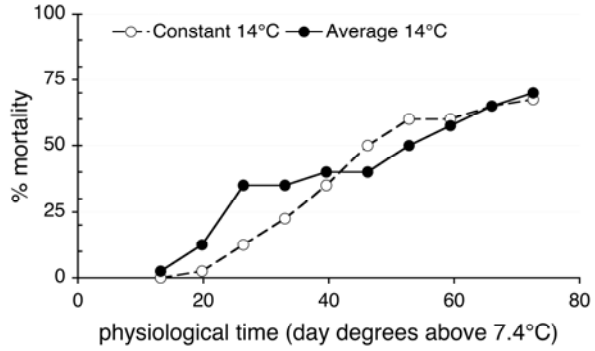

Figure 6. Per cent mortality of *P. xylostella* larvae (susceptible Waite population) exposed to 1 ppm of fipronil. Larvae were held at either constant 14˚C or mean 14˚C (cycling between 20˚C and 9˚C in 24 h period)

in southern Australia, with *Crocidolomia pavonana*, *Hellula undalis*, *Spodoptera litura* and *Helicoverpa* spp. being significant pests in spring and summer, as well as *P. xylostella* and *Pieris rapae* (the main pests in southern states). Consequently, endosulfan was used much more extensively in the 1980s and 1990s in Queensland than in the southern states.

The slow action of fipronil, coupled with the reduced susceptibility of Queensland populations, contributed to the unease of Queensland growers who were unhappy with the level of control observed when fipronil was used in the Lockyer Valley in the winter of 1998 (mean temperature at Gatton Research Station between June-August was 14.1°C).

The positive temperature coefficient found for fipronil contrasted with the well-recognised negative temperature coefficient of most pyrethroids (Scott 1995). Among the new chemistries registered for control of *Plutella* in *Brassica* crops, only spinosad has been shown to have a negative temperature coefficient (Musser and Shelton 2005). Indoxacarb (Wing et al. 2005); flubendiamide (Hirooka et al. 2007) and abamectin (Boina et al. 2009) have all been shown to have a positive temperature coefficient.

Although fipronil was welcomed as the first of several new chemistries registered for control of *P. xylostella* (L.), on *Brassica* vegetables in Australia at a time when pyrethroid insecticides were failing, its use has now declined as other products such as spinosad, indoxacarb and emamectin benzoate became available to growers. These other insecticides have less severe effects on natural enemies of *P. xylostella* (Williams et al. 2003; Shi et al. 2004) and so were considered by the growers to be more appropriate for use in integrated systems. A risk of future development of insecticide resistance to the newer chemistries other than fipronil would occur if one of these compounds is found to be more efficacious than the others, leading to overuse. This is a good argument for continued adherence to the two-window IRM strategy. In fact, Rahmann et al. (2010) observed elevated levels of tolerance (attributed to inducible tolerance mechanisms) to emamectin benzoate in Australian field populations of *P. xylostella* after frequent use of the insecticide.

CONCLUSIONS

1. Field populations of *P. xylostella*, primarily from Queensland, showed reduced susceptibility to fipronil compared to susceptible populations, prior to the field use of fipronil in 1997. These findings were confirmed with further detailed testing in the years following the registration of fipronil for use against *P. xylostella* in *Brassica* vegetable crops.

2. Bioassay data revealed an association between reduced susceptibility to fipronil and resistance to endosulfan. Queensland growers traditionally had used endosulfan more frequently than southern growers because of the greater diversity of lepidopteran pests of *Brassica* vegetables in Queensland.

3. Fipronil had a positive temperature coefficient, with efficacy found to be highest at 28°C against the susceptible Waite population. The slower time of action observed by Queensland growers during winter could largely be accounted for by lower development rates of *P. xylostella* larvae at low temperatures. This was demonstrated in the temperature-mortality studies by using a physiological time scale (lower temperature threshold of 7.4°C). No difference in mortality was observed when *P. xylostella* larvae were exposed to fipronil (1 ppm) at 14°C, at either constant or fluctuating temperatures cycling between 20°C and 9°C.

4. The perceived poor field performance of fipronil in winter in Queensland is likely to have been influenced

by the inherent lower susceptibility of these populations, coupled with the slower action of the insecticide at reduced temperatures.

5. Fipronil is no longer widely used by Australian vegetable *Brassica* growers, but its decline in use was largely related to issues other than resistance as growers and consultants prefer other insecticide chemistries, now available, that were more effective and that had lower impact on the suite of natural enemies in the field.

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Diamondback moth resistance to commonly used insecticides in Fiji

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ABSTRACT
Plutella xylostella is a serious pest of cabbage crops in Fiji and bi-weekly applications of synthetic insecticides are commonplace. In 2009, 95% of cabbage farmers in the Sigatoka valley, on the main island of Viti Levu, applied synthetic pyrethroids, 67% used indoxacarb, 28% used lufenuron, 23% used organophosphates but only 8% used *Bacillus thuringiensis* (Bt). In 2008 a population of *P. xylostella* from the Sigatoka valley exhibited significant resistance to deltamethrin (resistance ratio (RR) = 487) and indoxacarb (RR=56) but was susceptible to both lufenuron and Bt. In 2009 separate populations of *P. xylostella* were established from the upper, mid and lower regions of the Sigatoka valley, from Koronivia on the east coast of Viti Levu and from Labassa on the neighboring island of Vanua Levu. The Sigatoka valley populations were resistant to deltamethrin (RR ranged 41-191) and indoxacarb (RR ranged 40-89) but susceptible to lufenuron and Bt. The Labassa population was resistant to deltamethrin (RR= 150), moderately resistant to indoxacarb (RR=15) but susceptible to lufenuron and Bt, while the Koronivia population was resistant to lufenuron (RR=29), moderately resistant to indoxacarb (RR=12) but susceptible to deltamethrin and Bt. In the absence of selection in the laboratory, resistance to both deltamethrin and indoxacarb declined and disappeared completely within 10-15 generations, indicating that resistance to these insecticides confers a fitness cost. However, laboratory selection of field populations with either deltamethrin or indoxacarb caused resistance levels to increase dramatically (deltamethrin RR=466 and indoxacarb RR=892 following selection for three generations with deltamethrin and indoxacarb respectively). There was significant cross resistance between deltamethrin and indoxacarb but resistance to these insecticides did not affect the susceptibility of insects to lufenuron, Bt or the anthranilic diamide rynaxypyr, which was uniformly effective against all populations tested. Results are discussed in the context of the historic use of insecticides in Fiji and the development and implementation of an insecticide resistance management strategy is considered.

Keywords
Insecticide resistance; pyrethroids; indoxacarb; *Bacillus thuringiensis*; rynaxypyr.

INTRODUCTION
Although it is one of the most developed economies in the Pacific, Fiji retains a large subsistence agricultural sector. Approximately 13.8% of the total land area of 1,827,000 ha is devoted to arable and permanent crop production and 36.2% of the 854,000 inhabitants are involved in agriculture (FAOSTAT 2010). In Fiji, the annual market value of vegetable crops is estimated to be FJD$10.2M; leafy vegetables account for FJD$2.5M. Cabbages, which represent 80% of leafy vegetable production, have an annual value of approximately FJD$2M (Furlong, 2005; Furlong, 2010).

Commercial *Brassica* crops, especially "head" or "English" cabbage (*Brassica oleracea*) and Chinese cabbage (*Brassica rapa*), are extremely important to the rural economies in Fiji. Increasingly, as traditional vegetables such as bele (*Abelmoschus esculenta*), taro (*Colocasia esculenta*) and fern leaves (ota) become less available in local markets, *Brassica* crops are becoming the most important leafy vegetable in the local diet. The booming tourist trade in Fiji has created further demand for high quality *Brassica* vegetables. Currently, English cabbage imports are significant and increasing local supply for import substitution is an important development goal for the local economy.

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is a destructive pest of *Brassica* crops in Fiji (Waterhouse and Norris, 1987) and together with the large cabbage moth, *Crocidolomia pavonana* F. (Lepidoptera: Crambidae), it represents the major constraint to production. The Sigatoka Valley, on the main island of Viti Levu, is Fiji's major vegetable production area and the limited numbers of commercial insecticides which are readily available to farmers are used intensively. Traditionally insect pest management in *Brassica* crops in the Sigatoka valley has relied solely on the calendar application of broad spectrum insecticides (Kfir 2003). In late 2009 a farmer survey in the area showed that insecticides continue to represent the only approach to insect pest control and that they are commonly applied to *Brassica* crops 2-3 times per week (Atumurirava, unpublished data). In the interviews 95% of respondents report that they used pyrethroid insecticides, 67% used indoxacarb but only 8% used *Bacillus thuringiensis* (Bt); a majority of farmers considered the pyrethroid insecticides that they used (principally deltamethrin) and indoxacarb to be less potent than in previous times but none reported total control failures in the field.

MATERIALS AND METHODS

Insecticides

Deltamethrin (Suncis®; 25g ai/kg), indoxacarb (Steward®; 200g ai/L) and rynaxypyr (Prevathon®; 50g ai/L) were supplied by AgChem Fiji Ltd. Lufenuron (Match®; 50g ai/L) was supplied by Morris & Hedstrum Fiji Ltd. and *Bacillus thuringiensis* subsp. *kurstaki* strain HD-1 (Dipel®DF; 54%) was supplied by Landmark Ltd., Australia. All commercial insecticide formulations were stored at room temperature.

Plants

Chinese cabbage, *Brassica rapa* var. chinensis Pak choy, was used in all experiments and for rearing the different *P. xylostella* strains. Seeds were sown into a mixture of river bank alluvial soil mixed with poultry manure in seed beds. When seedlings were four weeks old they were transplanted into potting bags filled with the same soil-manure medium and grown to the 7-8 leaf stage in a shade-house before use in bioassays or for maintaining the insect cultures.

Insects

Culture methods

All *P. xylostella* strains were reared separately at room temperature (25^{o}C). Adult moths were held in wooden framed muslin covered oviposition cages (45 x 45 x 45 cm) containing a fresh potted Chinese cabbage plant (7-8 leaf stage) and an adult food source (20% w/v aqueous honey solution). Plants were exposed to adult moths for 24 h and replaced daily. Each egg-laden plant was labeled with the *P. xylostella* strain and oviposition date; plants containing eggs of different strains were kept separately from each other. When the eggs on a plant hatched, the plant was cut at its base, transferred to a ventilated plastic box (10 x 20 x 30 cm) and covered with freshly excised Chinese cabbage leaves. Old dry leaves were removed daily and replaced with fresh leaves. When the insects developed to pupae they were carefully removed from the rearing box and stored in labeled Petri dishes (9cm diameter) before being used to maintain the culture.

P. xylostella *strains*

An insecticide-susceptible reference strain (Waite strain) was obtained from the South Australian Research and Development Institute, Adelaide, where it has been maintained for more than 200 generations without exposure to insecticides.

Between September and December 2008 a total of 830 *P. xylostella* larvae and pupae were collected from commercial cabbage crops in the lower and mid regions of the Sigatoka valley. All collections were mixed to form a composite population (SIGA stain).

In 2009 separate *P. xylostella* populations were established from collections of larvae and pupae from commercial cabbage farms located in the lower, mid and upper regions of the Sigatoka valley. Approximately 500 larvae and pupae were collected from lower valley sites (Sigatoka Lower Valley strain; SLV) on 22nd August, approximately 500 larvae and pupae were collected from mid valley sites (Sigatoka Mid Valley strain; SMV)) on 23rd September, and approximately 700 larvae and pupae were collected from upper valley sites (Sigatoka Upper Valley strain; SUV) on 26th September.

On 21st September approximately 400 *P. xylostella* larvae were collected from a Chinese cabbage field at the Fiji College of Agriculture Farm, Koronivia in the east of Viti Levu (Fiji College of Agriculture strain; FCA).

Finally a *P. xylostella* population was established from approximately 800 larvae and pupae collected from cabbage plots near Labassa (Labassa strain; LAB) on the island of Vanua Levu on 26th October.

Leaf-dip bioassays

A standard leaf disc bioassay method which was modified from previous studies (Furlong et al., 1994; Sayyed et al., 2000; Endersby et al., 2001; Endersby et al., 2008) was used for all test insecticides. Test-solutions of each insecticide were prepared from commercial formulations using distilled water and 0.03% Tween-80 as a surfactant. Leaf discs (4.8 cm diameter) were cut from the middle leaves of Chinese cabbage plants and then immersed into the test solution for 10 seconds; excess solution was allowed to drip off from the leaf discs for another 10 seconds and then treated leaf discs were carefully placed, abaxial surface uppermost, on corrugated aluminium foil to dry for 1 h. Control leaf discs were treated by immersion in distilled water containing surfactant (0.03% Tween-80) only and then drained and dried in the same manner as insecticide-treated leaf discs. Four leaf discs were treated with each test solution and the control. When dry, treated leaf discs were placed individually into Petri dishes (5cm diameter) lined with moist Whatman No.1 filter papers; 10 early 3rd instar larvae were then carefully introduced to each Petri dish. In a given bioassay six to seven test insecticide solutions and a Tween-80 control were tested. Petri dishes were taped together, placed in a ventilated plastic container and incubated at 25 (±2^{0}C) with 12:12 (L:D) Treated leaf discs were removed after 48 h and replaced with fresh untreated Chinese cabbage leaves. Assessment of mortality varied between test insecticides: mortality caused by deltamethrin, indoxacarb and rynaxypyr was assessed after 72 h, while mortality caused by lufenuron and Bt was assessed after 96 h. Mortality was determined by prodding each larva gently with a paint brush; any larva that did not respond to touch was regarded as dead.

Bioassays of field-collected P. xylostella *strains*

Each field-collected population of *P. xylostella* was bio-assayed with each of the test insecticides as soon as possible after the establishment of the population in the laboratory. The Waite population was simultaneously bioassayed with each test insecticide so that comparisons

of the relative potency of each insecticide against each field strain could be made.

Laboratory selection for resistance

At generation F_5 post field collection, the SLV population was divided into three subpopulations (n=400). One subpopulation was left unselected (SLV-US) and continued to be cultured as previously described. Larvae for a second subpopulation (SLV-DS) were selected by exposure to the LC_{50} of deltamethrin against the SLV strain at F_3 (0.9 ppm deltamethrin) and larvae for the third population (SLV-IS) were selected by exposure to the LC_{50} of indoxacarb against the SLV strain at F_3 (7.5 ppm indoxacarb). The method used for selection was identical to that used in standard bioassays. Pupae from each population which survived insecticide exposure were placed in an oviposition caged together and when adults eclosed they were supplied with a Chinese cabbage plant for oviposition. A minimum of 400 third instar larvae from the SLV-DS and SLV-IS populations were selected with deltamethrin and indoxacarb respectively at F_5, F_6 and F_7 while the SLV-US population was maintained in the absence of exposure to insecticide.

Susceptibility of selected and unselected P. xylostella *populations to test insecticides*

Prior to its division into three sub-populations the SLV population was bioassayed with deltamethrin, indoxacarb, lufenuron, Bt and rynaxypyr at generation F_5. A simultaneous series of bioassays tested the susceptibility of the corresponding generation of the Waite population to each insecticide. All bioassays were repeated with the SLV-US, SLV-DS and SLV-IS population at F_8, following three generations of insecticide selection of the SLV-DS and SLV-IS populations.

Data analysis

Bioassay mortality data was subject to logit analysis using Polo Plus version 1.0 (LeOra Software 2002) and the LC_{50}, its 95% confidence interval, and the slope (±SE) of the regression line estimated. Resistance ratios (RR) were determined by dividing LC_{50} of the given field population by LC_{50} of the susceptible Waite strain.

RESULTS AND DISCUSSION

Bioassays of field-collected P. xylostella *strains*

There was no evidence for resistance to deltamethrin in the FCA field population but all other field populations exhibited high levels of resistance to this insecticide when compared with the susceptible Waite population (Table 1). In 2009 most bioassays were conducted on the first generation of insects after field collection and the data are likely to accurately reflect the resistance status of insects in the field. It is noteworthy that resistance levels recorded for the SMV and SUV populations, which were collected from regions of the Sigatoka valley which are farmed less intensively and which are cooler than the lower region of the valley, are considerably lower than the RR for the SLV population. The SLV and SIGA populations were not bioassayed until generations F_3 and F_4, respectively, after field collection; however, these populations exhibited the highest degrees of deltamethrin resistance recorded and, due to the delay in the collection of the data, these may well be underestimates of the level of deltamethrin resistance in field populations.

Only populations collected from the Sigatoka valley (SIGA, SLV, SMV and SUV) exhibited high levels of resistance to indoxacarb when compared with the Waite population (Table 2) but the resistance ratios were all lower than those recorded for deltamethrin (Table 1). Conversely there was no evidence for resistance to lufenuron in any of the populations collected from the Sigatoka valley and only the FCA population exhibited resistance to this insecticide (Table 3). Similarly there was no evidence for resistance to Bt in any of the field populations collected during the study (Table 4). In the Sigatoka Valley 95% of *Brassica* farmers apply pyrethroids and 67% apply indoxacarb while only 22% use lufenuron and almost none use Bt. The patterns of resistance discovered in the various *P. xylostella* populations collected across Fiji reflect the intensity of use of the different insecticides indicating that local selection pressures are important determinants of insecticide resistance levels.

Susceptibility of selected and unselected P. xylostella *populations to test insecticides*

Selection of the SLV-DS and SLV-IS populations for three generations with deltamethrin and indoxacarb respectively, increased resistance levels to these insecticides markedly (Table 5). Although selection with deltamethrin resulted in cross resistance to indoxacarb, selection with indoxacarb did not confer resistance to deltamethrin (Table 5). Selection with either deltamethrin or indoxacarb had no effect on the susceptibility of these *P. xylostella* populations to lufenuron or Bt. All test populations, including the insecticide-susceptible Waite population, were equally susceptible to rynaxypyr, indicating that this new compound will be a useful addition to the limited number of insecticides available to Brassica farmers in Fiji. However, recent anecdotal reports of the development of resistance of *P. xylostella* to rynaxypyr in parts of southeast Asia due to its excessive use should serve as a salutary warning that the useful life of this potentially very useful insecticide will be compromised if it is not used strategically, ideally as part of a national or regional insecticide resistance management strategy.

Table 1: Susceptibility of six field-collected diamondback moth populations and the laboratory reference population to deltamethrin in leaf-dip bioassays.

Popn	Generation	n	LC50 (95% CI) [a]	slope (±SE) [b]	χ^2 (df)	RR (95% CI) [c]
SIGA	F_4	280	38.1 (16.8- 178.4)	1.19 (±0.23)*	14.0 (22)	487 (118-2000)
SUV	F_1	280	3.46 (2.05- 5.95)	1.60 (±0.26)*	5.4 (22)	41 (16- 101)
SMV	F_1	280	3.56 (1.91-7.17)	1.23 (±0.22)*	10.6 (22)	42 (16- 112)
SLV	F_3	280	16.2 (10.2-22.5)	5.37 (±1.50)	14.1 (21)	191 (84- 434)
FCA	F_1	280	0.278 (0.177-0.400)	2.71 (±0.43)*	17.5 (18)	3 (1-8)
LAB	F_1	280	12.8 (3.37- 895)	0.47 (±0.16)	14.1 (22)	150 (21- 1085)
WAITE	F_5	280	0.078 (0.018- 0.16)	1.67 (±0.37)	10.6 (22)	-
	F_{16}	280	0.085 (0.023-0.151)	2.18 (±0.57)	7.8 (13)	-

[a] Concentration of deltamethrin expressed in parts per million (ppm)
[b] Slopes for field-collected populations marked with an * are not significantly different (P>0.05) to the slope of the Waite population at the appropriate generation (SIGA, tested against Waite F_5; all other field populations tested against Waite F_{16}).
[c] RR= resistance ratio of given field population against the appropriate generation of the susceptible Waite population [calculated as lethal dose ratio at LC_{50} in Polo-Plus (LeOra Software 2002)]

Table 2: Susceptible of six field-collected diamondback moth populations and the laboratory reference population to indoxacarb in leaf-dip bioassays.

Popn	Generation	n	LC50 (95% CI) [a]	slope (±SE) [b]	χ^2 (df)	RR (95% CI) [c]
SIGA	F_4	320	2.83 (1.67-4.69)	2.18 (±0.26) *	36.8 (22)	56 (19.3-163.6)
SUV	F_1	320	4.91 (2.99-8.95)	1.77 (±0.27)	20.9 (18)	86 (27.1-273.4)
SMV	F_1	320	2.31 (1.55-3.44)	2.49 (±0.33) *	20.6 (18)	40 (13.2-123.5)
SLV	F_3	320	5.10 (2.40-7.56)	4.22 (±1.09) *	22.7 (18)	89 (29.0-275.7)
FCA	F_1	320	0.68 (0.44-1.00)	2.85 (±0.42) *	26.6 (25)	12 (3.85-39.0)
LAB	F_1	320	0.85 (0.50-1.31)	1.75(±0.25)	20.5 (22)	15 (4.64-47.8)
WAITE	F_5	320	0.050 (0.013-0.116)	1.20 (±0.23)	14.4 (18)	-
	F_{16}	320	0.056 (0.008- 0.155)	1.20 (±0.25)	19.8 (17)	-

[a] Concentration of indoxacarb expressed in parts per million (ppm)
[b] Slopes for field-collected populations marked with an * are not significantly different (P>0.05) to the slope of the Waite population at the appropriate generation (SIGA, tested against Waite F_5; all other field populations tested against Waite F_{16}).
[c] RR= resistance ratio of given field population against the appropriate generation of the susceptible Waite population [calculated as lethal dose ratio at LC_{50} in Polo-Plus (LeOra Software 2002)]

Table 3: Susceptibility of six field-collected diamondback moth populations and the laboratory reference population to lufenuron in leaf-dip bioassays.

Popn	Generation	n	LC50 (95% CI) [a]	slope (±SE) [b]	χ^2 (df)	RR (95% CI) [c]
SIGA	F_4	280	1.41 (0.98-1.96)	2.63 (±0.36)*	9.6 (18)	3 (1.73-5.98)
SUV	F_1	280	1.80 (0.86-3.09)	1.65(±0.28)*	15.5 (22)	4 (1.72-11.46)
SMV	F_1	280	0.88 (0.33-1.69)	1.23 (±0.22)*	13.7 (22)	2 (0.76- 6.31)
SLV	F_3	280	3.25 (1.61-6.02)	1.20 (±0.19)*	24.4 (22)	8 (3.19-20.22)
FCA	F_1	280	11.78 (5.75-24.79)	1.29 (±0.26)*	8.5 (22)	29 (10.75-79.03)
LAB	F_1	280	1.33 (0.67-2.25)	1.36 (±0.24)*	9.7 (18)	2 (1.29-8.30)
WAITE	F_5	280	0.44 (0.23-0.68)	2.12 (±0.36)	9.1 (18)	-
	F_{16}	280	0.40 (0.13-0.72)	1.95 (±0.45)	8.7 (14)	-

[a] Concentration of lufenuron expressed in parts per million (ppm)
[b] Slopes for field-collected populations marked with an * are not significantly different (P>0.05) to the slope of the Waite population at the appropriate generation (SIGA, tested against Waite F_5; all other field populations tested against Waite F_{16}).
[c] RR= resistance ratio of given field population against the appropriate generation of the susceptible Waite population [calculated as lethal dose ratio at LC_{50} in Polo-Plus (LeOra Software 2002)]

Table 4: Susceptible of six field-collected diamondback moth populations and the laboratory reference population to Bt in leaf-dip bioassays.

Popn	Generation	n	LC50 (95% CI)	slope (±SE) [a]	χ^2 (df)	RR (95% CI) [b]
SIGA	F_4	280	0.0045 (0.0028-0.0079)	2.21(±0.32) *	29.9(18)	8 (3.46-19.51)
SUV	F_1	280	0.0014 (0.0008-0.0022)	2.072(±0.32) *	27.8(22)	2 (1.07-3.89)
SMV	F_1	280	0.0026 (0.0015-0.004)	2.008(±0.332) *	18.9(22)	4 (1.87-7.38)
SLV	F_3	280	0.0068 (0.0037-0.0115)	2.502(±0.394) *	31.1(22)	10 (5.07-18.72)
FCA	F_1	280	0.0018 (0.0010-0.0029)	2.218(±0.392) *	23.6(18)	3 (1.37-4.95)
LAB	F_1	280	0.0029 (0.0019-0.0046)	2.545(±0.325) *	37.8(22)	4 (2.34-7.58)
WAITE	F_5	280	0.0006 (0.0002-0.0010)	1.669(±0.390)	17.9(18)	-
	F_{16}	280	0.0007 (0.0003-0.0010)	2.411(±0.470)	15.4(22)	-

[a] Slopes for field collected populations marked with an * are not significantly different (P>0.05) to the slope of the Waite population at the appropriate generation (SIGA, tested against Waite F_5; all other field populations tested against Waite F_{16}).
[b] RR= resistance ratio of given field population against the appropriate generation of the susceptible Waite population [calculated as lethal dose ratio at LC_{50} in Polo-Plus (LeOra Software 2002)]

Table 5: Resistance ratios of deltamethrin-selected (SLV-DS), indoxacarb-selected (SLV-IS) and unselected (SLV-US) populations of the Sigatoka Lower Valley population to deltamethrin, indoxacarb, lufenuron and Bt and the susceptibility of these populations and the Waite population to rynaxypyr in leaf dip bio-assays.

	Resistance ratio (RR)[a]					Response to rynaxypyr	
Popn	Generation	Deltamethrin	Indoxacarb	Lufenuron	Bt	LC50 (95% CI)	Slope (±SE)
SLV-US	F_5	90	667	8	10	0.19 (0.09-0.31)	2.44 (±0.48)
SLV-US	F_8	39	42	8	8	-	-
SLV-DS	F_8	384	466	8	5	0.16 (0.10-0.22)	3.69 (±0.73)
SLV-IS	F_8	77	892	7	4	0.14 (0.09-0.19)	2.87 (±0.53)
Waite	F_{20}	-	-	-	-	0.15 (0.09-0.22)	3.27 (±0.68)

[a] RR= resistance ratio of given field population against the appropriate generation of the susceptible Waite population [calculated as lethal dose ratio at LC_{50} in Polo-Plus (LeOra Software 2002)]

CONCLUSIONS

- Resistance to deltamethrin and indoxacarb is widespread in Fiji but specific patterns of resistance are highly localized and reflect recent insecticide use.
- Deltamethrin and indoxacarb resistance decline rapidly in the absence of selection, indicating that fitness costs are associated with the currently unidentified resistance mechanisms.
- Selection of resistance to deltamethrin conferred high levels of resistance to indoxacarb but selection of resistance to indoxacarb served merely to maintain levels of resistance to deltamethrin.
- Laboratory-selected populations with extremely high levels of resistance to deltamethrin and indoxacarb retained susceptibility to Bt and were extremely susceptible to rynaxypyr. These IPM-compatible insecticides have the potential to make a significant contribution to sustainable IPM strategies for the management of *P. xylostella* and other *Brassica* pests in Fiji but their use will need to be managed effectively to maintain their efficacy.

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Spinetoram, a new spinosyn insecticide for managing diamondback moth and other insect pests of crucifers

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ABSTRACT
Spinetoram is an effective new tool for managing many crucifer insect pests. It is a new active ingredient in the spinosyn class of chemistry and is created by making two chemical modifications to naturally-occurring spinosyns. These modifications increase insecticidal activity and photostability. Spinetoram was reviewed under the US EPA Reduced Risk Pesticide Initiative and received a Presidential Green Chemistry Challenge Award in 2008.

Spinetoram has low toxicity to mammals and other non-target organisms such as birds and fish. It does not accumulate in the environment because it is rapidly degraded in soil and natural surface waters. Spinetoram has minimal effects on most beneficial arthropods and has an excellent fit in crucifer IPM programs.

The efficacy of spinetoram against *Plutella xylostella*, *Trichoplusia ni*, *Spodoptera exigua*, *Pieris* spp., and other crucifer pests has been demonstrated in field trials and under conditions of commercial use around the world. Use rates range from 9 to 88 grams ai per hectare, depending on crucifer pest species and geographical area. Spinetoram is safe to all crucifer crops. Spinetoram has the same unique mode of action as spinosad (IRAC Group 5). It activates certain nicotinic acetylcholine receptors which excites the insect central nervous system, causing paralysis and death of pest insects. Because spinetoram works directly on the insect nervous system, it is fast-acting. Larvae stop feeding and crawling within minutes of first exposure, and death occurs within 24 to 72 hours.

The product rotation programs practiced by growers in many parts of the world have been very effective in maintaining or restoring the susceptibility of *Plutella xylostella* to the spinosyns. Dow AgroSciences promotes and supports resistance management programs that rotate insecticides with different modes of action. Rotating multiple insecticide modes of action is needed to maintain effective and sustainable IPM programs for crucifer crops.

Keywords
Spinetoram, spinosad, spinosyn insecticide

INTRODUCTION
Spinetoram (Figure 1) is a new and highly effective insecticide for managing crucifer insect pests. It is the second active ingredient in the spinosyn class of insecticidal chemistry.

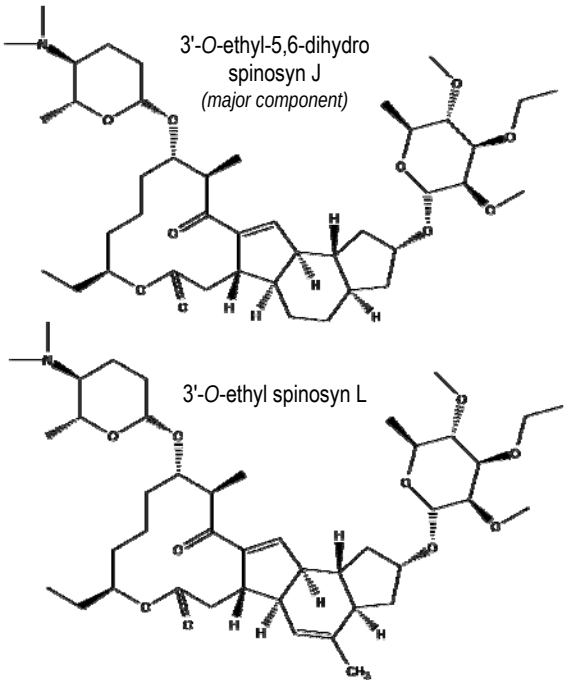

Figure 1. The two components of spinetoram.

Spinetoram was discovered with the innovative use of "artificial intelligence" (Dripps *et al*., 2008). *Saccharopolyspora spinosa*, a soil bacterium discovered in 1986, primarily produces spinosyns A and D, the components of spinosad, but also produces small amounts of 21 additional natural spinosyns. All of the spinosyns have rhamnose and forosamine sugars attached to a central tetracyclic lactone, but vary by the presence or absence of methyl groups at 8 positions around the sugars and lactone. These natural spinosyns vary in insecticidal activity and this "structure-activity" information was

used to find a way to improve on spinosyns A and D (Sparks, *et al*., 2008). An "artificial neural network" model, a form of artificial intelligence, was used to understand the structure-activity relationships among the natural spinosyns and predict more active structures. The artificial neural network analysis predicted that adding a methyl group to a specific position on the rhamnose sugar would greatly increase insecticidal activity. Other research determined that eliminating a double bond in the lactone increases stability in sunlight. Together, these chemical changes make spinetoram significantly more active and longer lasting than spinosad, without changing other attributes such as low mammalian toxicity and short persistence in the environment.

Spinetoram is created by chemically-modifying two natural spinosyns (Dripps *et al*., 2008). Spinosyns J and L are produced by fermentation with *Saccharopolyspora spinosa*. Spinosyns J and L then undergo two chemical reaction steps to create spinetoram. Spinetoram formulations for vegetables include a 60 gai/L SC (suspension concentrate), with the brand names Exalt™ and Endure™, and a 120 gai/L SC, with the brand name Radiant®.

Spinetoram was reviewed under the US EPA Reduced Risk Pesticide Initiative and received registration in the United States in 2007. In addition to the United States, spinetoram is currently registered in Canada, Mexico, China, and 23 other countries. Many additional registrations are pending around the world. Spinetoram was honored with the 2008 Presidential Green Chemistry Challenge Designing Greener Chemicals Award. This award recognized the innovative research approach used to discover spinetoram, its environmental benefits, and its "green", fermentation-based manufacturing process.

Regulatory profile

Like spinosad, spinetoram has a very favorable regulatory profile (Chloridis *et al*., 2007). It has low toxicity to mammals and is not mutagenic, teratogenic, or oncogenic. Spinetoram also has low toxicity to non-target organisms such as birds and fish. Spinetoram will have minimal environmental impact; it is rapidly biodegraded in soil, rapidly degraded in natural surface waters, and is essentially non-volatile. All spinetoram formulations are low in volatile organic compounds (VOCs).

Spinetoram has minimal effects on most beneficial arthropods and has an excellent fit in IPM programs. This is shown in a field trial on eggplant conducted in Mississippi, USA in 2005 (Figure 2). The total number of Neuroptera, Coccinellidae, Nabidae, Carabidae, and *Geocori*s spp. were sampled following applications of spinetoram (70 gai/ha), spinosad (70 gai/ha), or lambda-cyhalothrin (28 gai/ha). At 3 days after treatment, the total number of beneficial insects in the plots treated with lambda-cyhalothrin was reduced by almost 60% and remained well below the untreated plots at 7 and 14 days after application. In contrast, the total number of beneficial insects in the plots treated with spinetoram, like those treated with spinosad, was equal to or greater than the numbers of beneficial insects in the untreated plots at 3, 7, and 14 days after application.

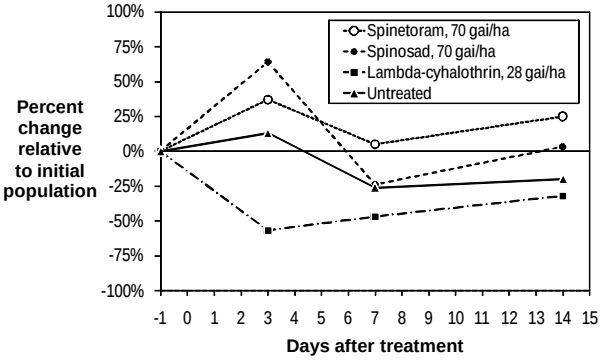

Figure 2. Effect of spinetoram and spinosad on the beneficial arthropod complex in eggplant.

Mode of action

Spinetoram has the same mode of action as spinosad (IRAC Group 5); no other insecticides share this mode of action. Spinetoram and spinosad activate unique nicotinic acetylcholine (nACh) receptors that excite the insect central nervous system, leading to paralysis and death of insect pests (Crouse, *et al*., 2007). Spinetoram and spinosad do not interact with any other known insecticide target sites, such as the target sites for avermectins, chloronicotinyls, pyrethroids, fiproles, organophosphates and carbamates, cyclodienes, or diamides. Because spinetoram works directly on the insect nervous system, it acts very quickly. Intoxicated larvae stop feeding and crawling within minutes of first exposure. This is followed by paralysis, weak tremors, and loss of body fluids. Intoxicated insects are unable to metabolize spinetoram or spinosad and do not recover. Death of treated insects occurs within 24 to 72 hours.

Efficacy

At use rates of 9 to 88 gai/ha, depending on pest species and geographical area, spinetoram provides excellent control of *Plutella xylostella* (diamondback moth), *Trichoplusia ni* (cabbage looper), *Spodoptera exigua* (beet armyworm), *Pieris* spp. (cabbageworms), and other insect pests in cruciferous crops. No crop injury has ever been observed following a spinetoram application.

Between 2005 and 2009, eleven field trials were conducted by universities and research institutes in China to compare the efficacy of spinetoram and indoxacarb for control of *Plutella xylostella* (Figure 3). A single application was made and the numbers of surviving *P. xylostella* larvae were recorded at five time points from 1 day to 10 days after application. The number of live larvae observed in each treated plot was used to calculate percent control relative to the number of live larvae in the untreated plots. Spinetoram at 13 to 38 gai/ha provided 68% to 77% control of *P. xylostella* larvae at 1 day after application. The highest levels of control (>90%) were obtained at 3 to 5 days after application. At 10 days after application, control remained at 77% to 86%. Indoxacarb at 36 gai/ha provided numerically lower knockdown of *P. xylostella* than the spinetoram treatments at 1 to 3 days after application and numerically lower residual control at 7 to 10 days after application.

Spinetoram is also highly effective against *Spodoptera exigua*, another key insect pest of crucifer crops. Figure 4 summarizes the results of six field studies conducted in China during 2005-2009 that compared the efficacy of spinetoram at 13 to 38 gai/ha to chlorfenapyr at 60 gai/ha against *S. exigua* larvae on cabbage. The same experimental methods were used in these trials as those used in the *Plutella xylostella* trials described above. Control of *S. exigua* obtained with spinetoram at 23 to 38 gai/ha was similar to the chlorfenapyr treatment from 1 day after application to 8 days after application. Spinetoram at 13 to 18 gai/ha was also effective, providing more than 80% control up to 5 days after application.

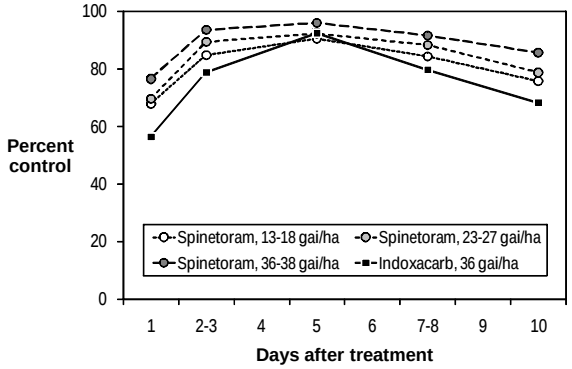

Figure 3. Efficacy of spinetoram against *Plutella xylostella* larvae on cabbage, 2005-2009, China; average of 11 field trials.

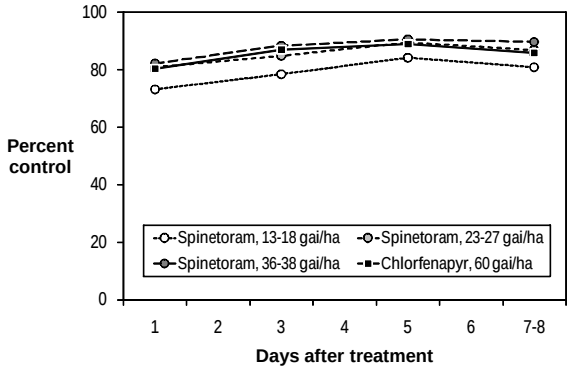

Figure 4. Efficacy of spinetoram against *Spodoptera exigua* larvae on cabbage, 2005-2009, China; average of 6 field trials.

In a 2008 trial on broccoli conducted in Arizona by Dr. John Palumbo of the University of Arizona, spinetoram at 44 gai/ha was compared to rynaxypyr at 73 gai/ha, and rynaxypyr+lambda-cyhalothrin at 52+26 gai/ha (Figure 5). Two applications were made 14 days apart. The cumulative number of *Trichoplusia ni* larvae per plant in the untreated plots reached 5.7 after 28 days (14 days after the second application). Spinetoram and rynaxypyr performed very well, reaching cumulative totals of 0.56 and 0.57 larvae per plant, respectively. The mixture of rynaxypyr+lambda-cyhalothrin was somewhat less effective, reaching a cumulative total of 1.53 larvae per plant.

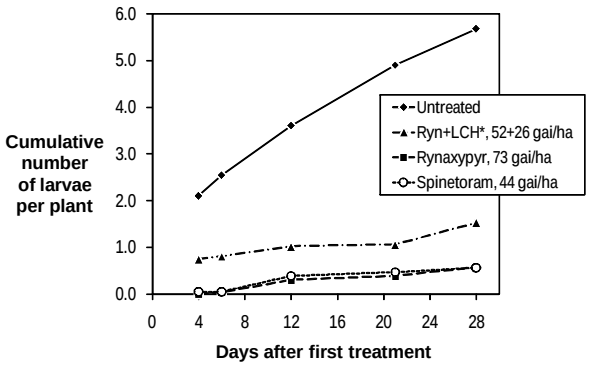

Figure 5. Efficacy of spinetoram against *Trichoplusia ni* on broccoli, 2008, Arizona, USA.

**** Ryn+LCH = rynaxypyr +lambda-cyhalothrin premix.***

Figure 6. Protection of cabbage against *Plutella xylostella*, *Trichoplusia ni*, and *Pieris brassicae* feeding damage with spinetoram, 2007, Mississippi, USA.

Spinetoram, 31 gai/ha (upper photo); Flubendiamide 35, gai/ha, (center photo); Untreated (lower photo).

In a 2007 trial on cabbage conducted in Mississippi by Dow AgroSciences researchers, spinetoram at 31 gai/ha was compared to flubendiamide at 35 gai/ha. The feeding damage caused by a complex of *Plutella xylostella*,

Trichoplusia ni, and *Pieris brassicae* larvae was assessed at 3, 7, and 14 days after the fourth application of each insecticide. Percent defoliation was estimated visually. Quality was assessed using Greene's scale, where a score of 1 equals no damage and 6 equals heavy damage to the wrapper leaves with significant scarring of the head leaves (Greene *et al*., 1969). Scores below 3 indicate damage only to the wrapper leaves. Cabbage heads with a score of 3 or less are considered to be marketable.

There was very heavy pest pressure in this trial, as can be seen in the photograph of an untreated plot in Figure 6. Cabbages collected from the untreated plots had an average quality score of 6.0 at 3, 7, and 14 days after fourth application, and defoliation increased from 70.3% to 74.9%, and finally reached 78.5% over this time period. Cabbages from the spinetoram plots had average quality scores of 2.3, 2.3, and 4.0 at 3, 7, and 14 days after the fourth application, and defoliation levels of 1.9%, 1.9%, and 8.8%. Cabbages from the flubendiamide plots had average quality scores of 2.8, 3.0, and 4.3 and defoliation levels of 2.1%, 3.1%, and 8.7%. There was no statistical difference in the quality scores or percent defoliation between spinetoram at 31 gai/ha and flubendiamide at 35 gai/ha.

Speed of action

We examined the speed of action of several new insecticides in a 2009 laboratory study. The insecticides and concentrations tested were: flubendiamide at 115 ppm (equivalent to 35 gai/ha at a 300 L/ha spray volume), indoxacarb at 265 ppm (80 gai/ha), rynaxypyr at 90 ppm (27 gai/ha), and spinetoram at 80 ppm (24 gai/ha). Commercial formulations available in the USA were tested. Potted cabbage plants were sprayed with a track sprayer. After the spray residue dried, leaf disks were punched from the treated leaves and infested with second instars of *Plutella xylostella* or *Trichoplusia ni*. The study was replicated 5 times and a total of 60 larvae of each species were tested at each concentration. At 1, 2, 5, 8, 18, 24, 48, and 72 hours of exposure, the larvae were observed and scored as "normal" (no visible effects), "affected" (uncoordinated, disoriented, or slow movement; or other symptoms of intoxication), or "dead" (no response when touched).

All four insecticides killed or intoxicated at least 80% of *Plutella xylostella* and *Trichoplusia ni* larvae within 72 hours (Figure 7). Spinetoram had the most rapid effects on both species. The percentage of *P. xylostella* larvae killed or affected by spinetoram was 38% at 2 hours, 82% at 5 hours, and 93% at 8 hours. The percentage of *P. xylostella* larvae killed or affected by rynaxypyr, flubendiamide, or indoxacarb ranged from 0% to 15% at 2 hours, 3% to 47% at 5 hours, and 15% to 67% at 8 hours. The percentage of *T. ni* larvae killed or affected by spinetoram was 27% at 2 hours, 58% at 5 hours, and 80% at 8 hours. The percentage of *T. ni* larvae killed or affected by rynaxypyr, flubendiamide, or indoxacarb ranged from 3% to 15% at 2 hours, 42% to 50% at 5 hours, and 50% to 78% at 8 hours.

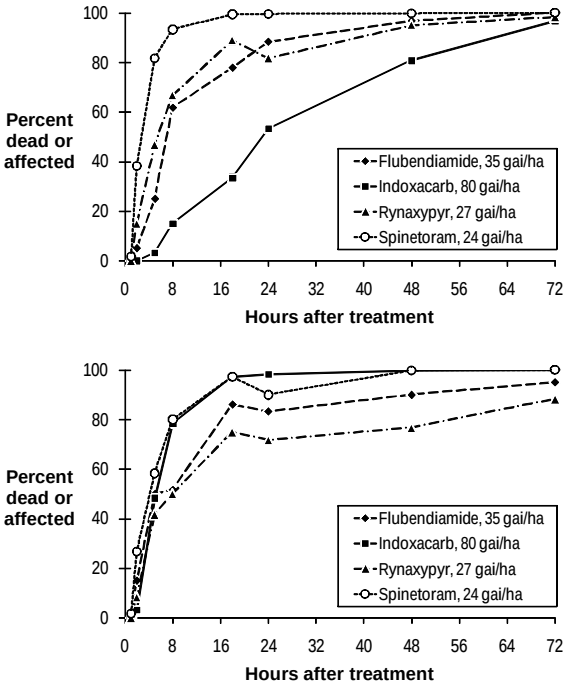

Figure 7. Speed of action of spinetoram against *Plutella xylostella* larvae (upper graph) and *Trichoplusia ni* larvae (lower graph).

Resistance management

Because spinetoram and spinosad share the same mode of action (IRAC Group 5), it is very important not to rotate spinosad and spinetoram with each other. Dow AgroSciences strongly supports proper rotation of the spinosyn insecticides with products having different modes of action, and is committed to all effective and proactive resistance management tactics that will maintain the effectiveness of spinetoram, spinosad, and other insecticides for all growers.

In a few cases, growers have not followed the advice of rotating insecticide products and have overused spinosad. Dow AgroSciences has gone as far as suspending some uses in order to preserve the efficacy of spinosyn products for the majority of growers who follow resistance management practices. This happened in 2006 in Georgia, USA. Several collard growers continued to overuse spinosad to control *Plutella xylostella* after being asked repeatedly to follow the resistance management guidelines on the product label. This overuse threatened the efficacy of spinosad in other crucifer crops. Dow AgroSciences suspended spinosad use on collards in Georgia to protect the efficacy of the spinosyns for the majority of crucifer growers in that state. As a result of this action, spinosad and now spinetoram continue to be very effective against *P. xylostella* in Georgia.

In most areas around the world, the insecticide rotation programs practiced by growers and promoted by Dow AgroSciences have been very effective in maintaining or restoring the susceptibility of *Plutella xylostella* to the spinosyn insecticides.

In Mexico, Dow AgroSciences monitored the susceptibility of *Plutella xylostella* to spinosad in the Bajio and

Norte del Estado areas of Guanajuato State between 1998 and 2007. Every 1 to 3 years, larvae were collected from the field and sent to Dr. Anthony Shelton at Cornell University for bioassay. F_1 or F_2 offspring were used for bioassays. Five cabbage leaf discs were dipped in each spinosad solution for 10 seconds, allowed to air-dry, and placed in a 30 mL plastic cup. Twenty *P. xylostella* second instars were introduced onto each leaf disk and mortality was determined after 72 hours.

Year to year variations in spinosad LC_{50} values were observed for these populations between 1998 and 2007, but no lasting changes occurred (Figure 8). The higher spinosad LC_{50} values observed in 2000 and 2006 triggered increased resistance management efforts by Dow AgroSciences and INIFAP, and these efforts successfully restored *P. xylostella* susceptibility to spinosad. *P. xylostella* monitoring bioassays after 2007 are being done by Dr. Rafael Bujanos of INIFAP. Spinetoram and spinosad efficacy against *P. xylostella* remains very high in Guanajuato as a result of Dr. Bujanos' efforts to promote insecticide rotation by crucifer growers in that region.

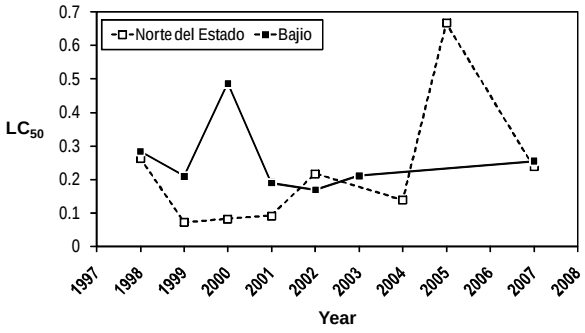

Figure 8. Susceptibility of *Plutella xylostella* populations to spinosad, 1998-2007, Guanajuato, Mexico.

In Taiwan, Dow AgroSciences has conducted bioassays to monitor *Plutella xylostella* susceptibility to spinosad at its Taiwan Agricultural Research Center in Pingtung since 2000. *P. xylostella* larvae and pupae were collected every 1 to 3 years from crucifer fields in Kaohsiung County (southern Taiwan) and Chun-hwa County (central Taiwan). F_1 or F_2 offspring were used for bioassays. Three cabbage leaf discs were dipped in each spinosad solution, allowed to air-dry, and placed into Petri dishes. Ten *P. xylostella* second or third instars were introduced in each dish, and mortality was recorded after 72 hours.

The spinosad LC_{50} for the Kaohsiung County population increased from 0.6 ppm in 2000 to 5.6 ppm in 2001 and reached a peak of 104 ppm in 2002 (Figure 9). After 2002, *P. xylostella* increased in susceptibility to spinosad from one year to the next, with an LC_{50} of only 3.6 ppm being observed in 2010. A very similar trend was found for *P. xylostella* collected in Chun-hwa (Figure 9).

The changes in spinosad LC_{50} values were consistent with observed field performance of spinosad in Taiwan. Spinosad efficacy against *Plutella xylostella* decreased after two years of commercial use but then increased until a high level of control was restored in 2009. When first introduced into Taiwan in 2000, spinosad was very effective against *P. xylostella* and growers tended to use it excessively. The recovery of *P. xylostella* susceptibility to spinosad after 2002 was the result of continued emphasis by Dow AgroSciences on the importance of insecticide resistance management (IRM) tactics, the introduction of new insecticides with different modes of action, and the acceptance of IRM tactics by crucifer growers in Taiwan once they realized the consequences of spinosad resistance.

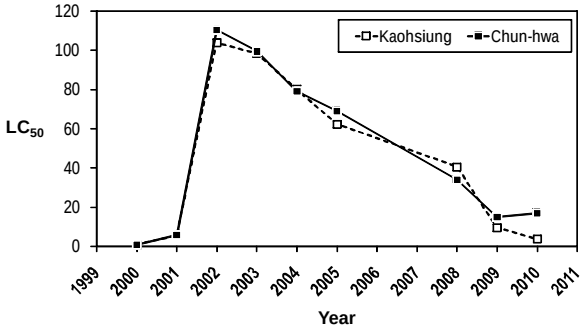

Figure 9. Susceptibility of *Plutella xylostella* populations to spinosad, 2000-2010, Taiwan.

Another successful story of managing *Plutella xylostella* susceptibility to spinosad occurred in Korea. Between 2001 and 2003, and resuming in 2008, *P. xylostella* larvae were collected on farms near JangSu (south-central Korea) and farms near PyungChang (northeastern Korea). Bioassays were conducted by Dr. Kil-Ha Kim of Chungbuk National University. F_2 or F_3 offspring were used for bioassays. Three Chinese cabbage leaf disks, 5 cm in diameter, were dipped in each spinosad solution for 30 seconds, allowed to air-dry for 1.5 hours, and then placed in a Petri dish. Ten *P. xylostella* third instars were introduced onto each leaf disk and mortality was assessed after 48 hours.

The susceptibility of *Plutella xylostella* populations in Korea has remained very high between 2001 and 2010, with the highest LC_{50} value being only 0.073 ppm for the JangSu population and 0.041 ppm for the PyungChang population (Figure 10). There has been very little change in the susceptibility of these populations to spinosad during 10 years of commercial use in Korea.

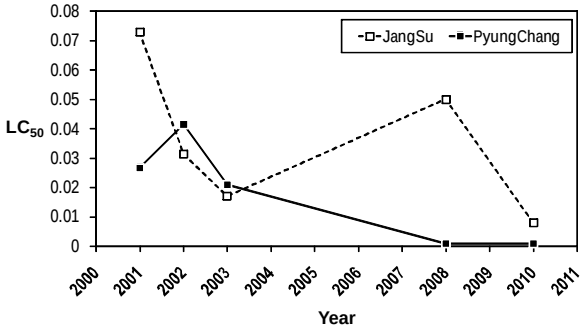

Figure 10. Susceptibility of *Plutella xylostella* populations to spinosad, 2001-2010, Korea.

SUMMARY

Spinetoram is a highly effective new spinosyn insecticide for the integrated management of lepidopterous pests in crucifer crops around the world. Spinetoram is created

by chemically modifying two naturally produced spinosyns (spinosyns J and L). Spinetoram is fast-acting, effective against a wide range of crucifer pests, provides good residual control, is safe to crops, and has minimal effects on most beneficial insects. Spinetoram has low toxicity to mammals and other non-target organisms, and is rapidly degraded in the environment.

Spinetoram is a Group 5 insecticide, and has the same mode of action as spinosad. Rotating insecticides with different modes of action is the key to maintaining effective and sustainable IPM programs for crucifer crops. The benefit of insecticide rotation and other insecticide resistance management (IRM) tactics in maintaining the efficacy of the spinosyn insecticides has been clearly demonstrated in the crucifer-producing areas of Mexico, Taiwan, and Korea.

Acknowledgements

We thank the many researchers and growers who evaluated spinetoram during its development. Our thanks also to Zhenwei Yao, Dow AgroSciences China, for his time and efforts in coordinating some field trials reported in this article.

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Insecticide resistance management: sharing the experience on diamondback moth in the Philippines

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ABSTRACT
Diamondback moth (*Plutella xylostella)* or DBM remains the major insect pest of cabbage and other crucifers in the Philippines since its outbreak in the mid-1960s.

The first report on DBM resistance to insecticides was in 1974, particularly to EPN and Mevinphos and to all kinds of insecticides including the microbial insecticide (*Bacillus thuringiensis)* thereafter. Multiple resistances to insecticides were also noted in 1976. In 1990, some farmers resorted to the use of cyanide-based materials in their desperation to contain the outbreak of DBM infestation, causing "Cyanide-Scare" in the process. Insect Growth Regulators (IGRs) were also used but generally short- lived. Meanwhile, the botanical insecticides that were claimed to be effective were not commercially available for farmer's use.

In 1989, the parasitoids *Diadegma* (for the highlands) and *Cotesia* (for the lowlands) were imported, mass reared and released in the field with almost 85% parasitization. In addition, an Integrated Pest Management-Farmer's Field School (IPM-FFS) was introduced and this became the core in managing insect resistance. In 2001, moderate DBM infestation was noted in untreated cabbage. Lately, infestations have been slight.

The Fertilizer and Pesticide Authority (FPA) also started to accredit researchers. The researchers, mostly coming from the academe, conduct efficacy trials, not only in the laboratory or experimental plots but also in farmer's fields while the Local Government Units (LGUs) conduct farmer training in collaboration with the academe and product developers. Judicious use of pesticides is emphasized by the industry staff during farmer training. Independently, sensitivity tests to monitor resistance levels against DBM were being conducted by some companies on their key products. They also decided to join together to combat resistance development.

There is a need to continue the IPM-FFS program, continue RDE activities and strengthen the network of service providers to sustain the population of DBM at low levels.

Keywords
cabbage, Cotesia, Diadegma, diamondback moth, insecticide resistance

INTRODUCTION
The diamondback moth (*Plutella xylostella* L.) or DBM, in the Philippines is more than 75 years old, as old as the first generation of farmers and scientists. DBM has been a major pest for almost 41 years (Capco 1959). DBM populations started to decline in 2001 (Cardona year of reference?), and untreated cabbage showed moderate damage, and in 2008 (Cardona year of reference?) infestations continued to decrease to the present with some plants only slightly damaged. When the presence of DBM in the Philippines will be 100 years old in 2027, will we celebrate in jubilation because she existed with us as a component of a balanced agro-ecosystem or will it be a celebration full of DBM Brigades similar to this workshop?

Diamondback moth is very much hated by the Filipino farmers, consumers and all other stakeholders in the vegetable industry like us. Nevertheless, it created a scenario such as this for different countries to meet, share knowledge and foster cooperation and peace. It has also created employment for millions of people around the world. So, while we "curse" the existence of DBM, let us be thankful that it has brought us all together in this wonderful country of Thailand.

We want the DBM to co-exist with us and this paper deals with the Philippine experience in coming up with a Filipinized DBM with fewer offspring causing only moderate to slight damage.

This paper reviewed relevant literatures from the time DBM was first recorded. The relevant data of a survey in Benguet on the current practices (first quarter of 2011) of cabbage growers and the kind of pesticides that are being sold for DBM by pesticide dealers were also presented.

Cabbage growing areas
Cabbage ranks second among the vegetable crops that are grown in the country. The area planted to cabbage in 2009 was 8,483 hectares. More than 85% is located in the cool highland areas in Benguet Province, the Salad Bowl of the Philippines. As of September 2010, the total volume of production was 134,305 MT amounting to PhP 606.16 M with a yield of 18.13 MT per hectare (BAS, 2011).

Figure 1 shows the cabbage growing areas in the Philippines. It is similar to the data presented by Magallona in 1985 but the areas expanded and included four more municipalities: Bauko in Mt. Province; and Kabayan, Kibungan and Mankayan in Benguet.

Insecticide resistance and pest outbreak

Several pest outbreaks occurred on various crops in the country and are generally attributed to the development of insecticide resistant strains. The first pest outbreak on vegetable crops was DBM on cabbage in the mid 1960's and the first report of insecticide resistance was to EPN and Mevinphos in 1974 (Barroga and Morallo-Rejesus, 1975). The pattern of pest outbreak seems to occur every ten years, not only with DBM but also insects on other crops such as potato-- aphids (1960s), thrips (1970s), potato tuber moth (1980s), and potato leafminer (1990s) (Molitas-Colting, 2003). The pest outbreaks are mainly due to monocropping and misuse of pesticides such as increased dosage, closer application interval, cocktail preparations and even use of banned insecticides such as Furadan for almost five decades (Sanchez et. al., 1968, and Padsungay and Ligat, 1998).

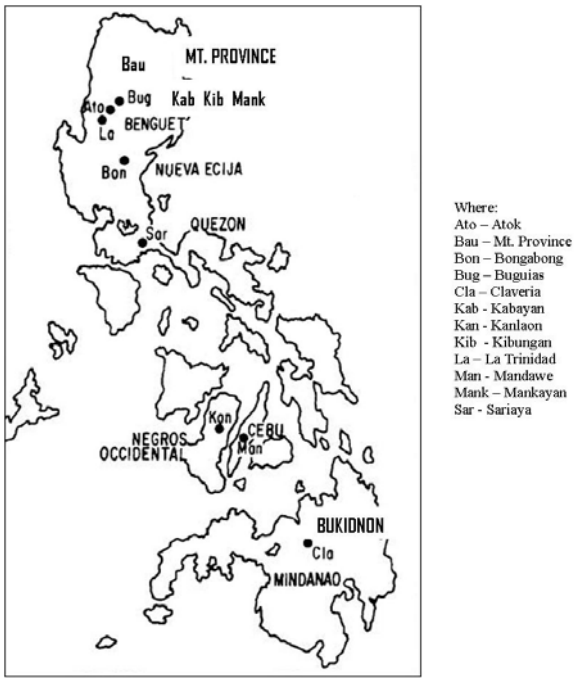

Fig. 1. Cabbage producing areas in the Philippines (adopted from Magallona, 1985)

Resistance was noted to almost all kinds of insecticides that were introduced, from the organochlorines, carbamates, organophosphates, pyrethroids, insect growth regulators (Barroga and Morallo-Rejesus, 1975); Cypermethrin (Quinit and Padrigo, 1980); Deltamethrin, Fenvalerate, Rotenone, Triazophos (Magallona et. al., 1982); Cartap (Verzola et. al., 1986); and Spinosad (Simong and Cardona, 2002). Multiple resistance was also noted to DDT, Carbaryl, Mevinphos, Malathion, Methyl Parathion, Diazinon and Dichlorvos (Barroga and Morallo-Rejesus, 1981). Resistance to *Bacillus thuringiensis (*Bt) was also observed (Boonsuwan and Hermano, 1977) only two years after it was found effective (Cadapan and Gabriel, 1975). The four kinds of Bts that were tested in 2001 were, however, found to be effective against DBM populations in Benguet (Sad-o and Molitas-Colting, 2001). It must also be the same in the case of the insect growth regulators (IGRs) and other kinds of insecticides that led to their withdrawal in the market.

To break DBM resistance to insecticides, other insecticides were tested, such as granular insecticides (Sanchez et. al., 1968), insect growth regulators (IGRs) (Tetangco-Fabellar, 1976), and botanical insecticides (Molitas-Colting and Mangali, 1994). There was also a search for biological control agents (Velasco, 1982). Farmers also did their own experiments but claimed that not one is effective. In fact, the majority of the studies on DBM dealt with insecticides and this may be one of the reasons why farmers do not have alternatives, and an indication that use of insecticides is the main strategy in managing the pest in the Philippines. Pesticide residues and health risks of farmers and consumers due to pesticides thus remain a concern from 1976 (Tejada et. al.) up to the present (Lu, 2010).

Few studies were made on comparative LD50 values but the development of resistance to insecticides could be attested to by the number of compounds which were effective when first introduced only to become ineffective later. It is surprising though that the insecticides that were sold in the 1960s are still available in the local market.

The Filipino farmer and consumer

The concept of insect resistance to insecticides among farmers must have evolved when they started experiencing the non-efficacy of insecticides that were previously effective despite the increase in dosage, cocktailing and closer spray interval. This must have been reinforced with actual field observations of "netted" and skeletonized cabbage plants and the occurrence of numerous larvae, pupae and flying moths.

Another factor that must have influenced a better understanding of how insects develop resistance to insecticides is their level of education. In 1998, Padsungay and Ligat noted that a majority of the IPM-FFS farmers surveyed (46%) were elementary school graduates, compared to the latest survey which showed that the majority (41%) are high school graduates, meaning they can read and understand simple instructions. Some (14%) completed a college degree in agriculture and therefore they have a better understanding about crop protection, crop physiology, ecology, etc. These agriculture graduates, though few, will surely help in convincing other farmers to practice IPM, considering that co-farmers as sources of information ranked third in the latest survey. Rola et. al. (2002) pointed out that graduates of FFS retain and share what they learn with fellow farmers. The survey likewise shows that building up of farmer relationships advocated in the Farmer's Field School (FFS) in 1992

still exist. The second source of information is radio, next to the extension workers of the pesticide industry. In the BSU-on-the-Air program in the highlands, almost 4,000 text messages are received annually by the anchor and about 50% of the inquiries were about what insecticide to use (pc. Dr. S. L. Kudan, anchor BSU-on-the-Air, 2011).

In addition, farmers conduct their own experiments and will continue to do so with the common belief of "To see is to believe" or "kita ko pati ko". They also mix one or two kinds of insecticides because they are unaware that insecticides of the same origin have different brand names. The use of generics on insecticide labels similar to the pharmaceuticals may help resolve this problem. The most dangerous "experiment" that they did was to use cyanide-based products in the 1990s. This caught the attention of the mass media and created a "Cyanide Scare" throughout the country and almost destroyed the viability of the cabbage industry as consumers started to refrain from buying vegetables. To bring back the confidence of consumers, various issuances were made by the government. The Bureau of Agricultural Research, for instance, posted on the web an article "Safe food and pesticide-free vegetables and fruits seen in markets" (Aquino, 2003). Another article followed, "Philippines: Cordillera farmers reduce pesticide use" (Inquirer.net, 2007). The positive thing about this incident is that consumers started to become more vigilant on the quality of farm products (Santos and Visaya, 1998). It prompted concerned sectors to look for alternative control measures such as the adoption of the IPM-DBM Technology of the Asian Vegetable Network while some farmers started to go into organic farming. Many organic farmers associations are now organized throughout the country with organic markets.

Despite the shift to organic farming, the high incidence of pesticide-related health risks and other concerns among farmers, consumers and the environment still exist as shown in a 2005 to 2010 survey (Lu, 2010). This was also noted in the latest survey where 91% of the farmers still use pesticides on cabbage, 85% use two or more kinds per season but they are used one after the other and with a longer interval from 7 to 21 days, unlike before.

Strategies

The ways in which farmers managed resistant DBM and other crop pests changed throughout the years as farm area increased, farm technology changed, environmental conditions changed, and consumer demands changed. The strategy can be summarized into four stages as follows: a) natural farming in the 1950s, b) pesticide-based farming system in the 1960s to 1980s, c) biocon-based IPM in the late 1980s to 1990s, and d) recently (2000s) - good agricultural practice or organic farming.

Natural farming includes cultural management and crop rotation which are still practiced at present. In the latest survey, 45% of the farmers practice crop rotation, 31% release Diadegma, 13% practice IPM while the remaining 13% do other practices such as organic farming.

From the 1960s to 1980s, because of the high profitability of growing cabbage, the area was expanded, monocropping was practiced and the use of insecticides increased as it is the most cumbersome control measures for insect pests. The pesticide-based farming can be seen in the studies conducted at Benguet State University (BSU) that assist farmers in Benguet. Studies on insecticides started in 1977 up to 2005 (Table 1). BSU started to offer entomology courses in 1976; the highest numbers were from 1977 to 1985 (Molitas-Colting, 2010). From 1986 to 1995, there were fewer studies and this is attributed to the intensive and nationwide adoption of biocon-based IPM-FFS. Studies during this period include the use of botanical insecticides and insect growth regulators which were later not utilized due to their limitations, and also the development of resistance to IGRs. There was an increase in 1996 to 2005 but during this period it was more on finding insecticides compatible to *Diadegma* and other natural enemies. The increase in the number of studies on insect biology and ecology in 2001 to 2005 is also in support of the IPM program. Between 2006 and 2010 there were no trials on insecticides when BSU started to shift to organic agriculture upon declaring itself a Pro-Organic University in 2004. In the field, however, farmers still use Methamidophos and the other insecticides that were introduced in the 1970s because these are still sold in the local market (Molitas-Colting et al., 2006 and Lu, 2010).

Table 1. Number of studies on DBM conducted at BSU, La Trinidad, Benguet from 1977 to 2010

TOPICS	'77-'80	'81-'90	'91-'00	'01-'10
Insecticides	21	20	8	16
IPM	1	6	18	23
Bio-ecology	4	8	18	29
Others	2	6	9	5

The concept of judicious use of pesticides was introduced by the pesticide industry sector in the 1980s not only to prevent the development of insecticide-resistant pest strains but to address other concerns such as farmers' health, pesticide residues in soil and water, the changing demand of consumers for "safe" or pesticide-free food crops, and decimation of natural enemies. Seminars and trainings were conducted nationwide in collaboration with concerned sectors.

Sustainable management of DBM only occurred in the 1990s when in 1989 the Philippines adopted the DBM biocon-based IPM-FFS program with the use of *Diadegma semiclausum* (in the highlands). Mass rearing and field release of *Diadegma* was done in BSU in 1990 and in two years, establishment of the parasitoid was achieved (Ahmend et al., 1994). There was considerable reduction on the use of pesticides in Atok, the place where DBM outbreaks usually occur, from 20 to 5 times during the rainy season and from 36 to 8 times during the dry season. The reduction of pesticide use is also

reflected on the pesticide volume imported and sold in Benguet and Mountain Province which declined to 20% in 1992 and 33% in 1994 but it led to the increase in the price of pesticides making it prohibitive for farmers to buy. Cabbage farmers were also able to harvest up to 25.2 tons/ha (Ali, 1995). In the lowlands, *Cotessia plutellae* was introduced (Morallo-Rejesus et al., 1996). The study of Cardona (1997) made considerable contributions to the success of the Diadegma-IPM-FFS program in Benguet. He did a comprehensive study on the biology of DBM and *Diadegma* and established techniques in mass rearing and field release, and in monitoring and evaluation of the efficacy of the parasitoids. He also identified insecticides that are compatible with *Diadegma.* In his latest study (Cardona, 2010), he noted that *Diadegma* efficacy remains high (85%) in areas where there was field releases and supplemented with Bt insecticide.

To encourage private-public partnership, the Department of Agriculture in 2004 launched a program on High Value Crops (HVCC). This empowers the private sector to expand investment in high value crops in partnership with farmers to foster economic growth and increase farmer's income with prime concern on consumer health and welfare. To sustain the partnership, the farmers have to produce products of good quality to enable them to join the high value or supermarket supply chain (Agribusiness Week, 2008).

Another strategy of the government is to give awards to outstanding farmers. The most progressive farmer among the first IPM-FFS participants was given an award. There is also an award for farmer scientists called "Siyentistang Magsasaka". This year, 2011, the Department of Agriculture gave the first award to a farmer doing outstanding work in organic farming. These farmers serve as "consultants" to co-farmers or even serve as focal persons to management, and their farms serve as demo farms.

The service providers

It took almost 50 years from the first outbreak of DBM for the service providers to realize that in developing sustainable management strategies for diamondback moth and other pests, preventing the development of insecticide resistant pest strains is a prime concern. While everyone focuses on IRM, nobody is "in charge"; there is no pest monitoring system or coordinating body especially when there is pest outbreak (Fig. 2). The "Cyanide Scare" in 1990 may not have occurred if there was an efficient pest monitoring system. The service providers are: the Department of Agriculture (DA), the Pesticide Industry, research development and extension (RDE) sector that includes the academe and research centers, the local government units, non-government organizations and people's organization.

The efficiency of the agricultural extension workers was improved by devolving them from the national government to the local government units (LGUs). To bring technologies to the farms, Farmers' Information Technology Services (FITS) that includes computer-based resources and texting were established in the LGUs by the Philippine Council for Agriculture,

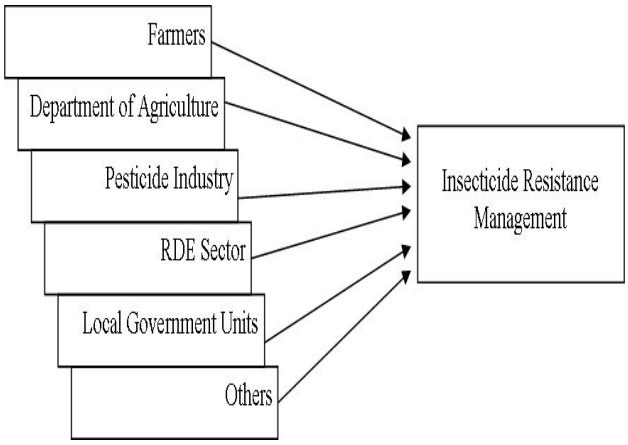

Fig. 2. How IRM is being addressed in the Philippines

Forestry and Resources Research Development (PCARRD) as information technology becomes available in the farming communities. An Open Academy by DA was also established in 2010, enabling farmers to study while at home. There is also a program that enhances the capabilities of researchers with the Fertilizer and Pesticide Authority (FPA) requiring the accreditation of researchers and a standard protocol in screening pesticides. On a limited scale, the state colleges and universities conduct RDE activities.

A nationwide campaign to shift to organic farming started in 2001 with the issuance of EO 481 for the Promotion and Development of Organic Agriculture. May was declared Organic Food Month (PP No. 137) in 2006 and the Philippine Organic Agriculture Road Map for 2007-2010 was also released. At present, there are now various institutions in the country that declare themselves pro-organic. Apart from BSU, there is an organic hospital, an organic municipality, an organic province, and many organic farmer associations and organic markets. The absence of pest outbreaks since then is attributed to the growing interest in organic farming. This was noted with the declining degree of infestation of DBM on cabbage from severe to slight damage in Luzon based on the latest personal communication with entomologists Dr. Arturo O. Manipon and Dr. Clarita P. Aganon of Central Luzon State University (Central Luzon area), and Dr. Pio O. Javier of the University of the Philippines Los Baños (Southern Luzon area).

In addition, the pesticide industry has conducted farmer training on judicious use of pesticides since the 1970s. In 2009, their network was strengthened with the establishment of CropLife Philippines. They support the government program in advocating good agricultural practices (GAP) and intensified their campaign on the management of empty containers of pesticides. Lately,

they organized the so called "Diamide Group" to re-tool/educate stakeholders on the principles and practice of insect/insecticide resistance management. Independently, they conduct sensitivity tests on their key products to monitor the levels of resistance of major pests, especially DBM.

With the current events, DBM seems not to be a problem pest. The declaration of DBM as a minor pest, however, needs further verification as the latest survey showed that a majority of farmers are still using insecticides. And in order to maintain such status, there is a need to strengthen the network of the service providers to include a monitoring and evaluation team and an overall coordinating body. The setting up of a Farm Text School may also be considered, with almost everyone, including farmers, having mobile phones and the government providing solar battery chargers to areas without electricity.

CONCLUSION

At 75, the DBM population in the Philippines must have been "Filipinized" and differs from her sisters in other countries: smaller, darker brown, and resistant to various insecticides. A comparative study on DBM strains may be considered.

While DBM populations have started to decrease, there is still a need to continue the biocon-based IPM-FFS for the sustainable management of the pest: re-establish the Diadegma/Cotesia Rearing House throughout the country, develop a farmer-friendly monitoring system, i.e. easier ways of sampling pest population and determining % of parasitism, retool farmers and other stakeholders on IPM, and introduce the concept of farm recording.

Strengthen also the network of service providers to facilitate pest monitoring and maximize resources. A "ready-to-go" QRS Team with QRS protocols in solving pest outbreak should likewise be established.

And, to keep the cabbage industry moving, IRM should be everybody's business before DBM reaches its centennial in 2027.

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Recent developments in management of diamondback moth in New Zealand

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ABSTRACT
Recent developments in management of diamondback moth (DBM), *Plutella xylostella*, in New Zealand include reassessment of the status of insecticide resistance, and updating the insecticide resistance management rotation strategy for DBM within the context of a revised integrated pest management (IPM) program for vegetable brassicas. The susceptibility of field populations to lambda-cyhalothrin, methamidophos, spinosad and indoxacarb collected in 15 growing areas from the four major brassica-growing regions was assessed approximately every two years from 2001 to 2008. Susceptibility levels were compared using a dose response leaf dip bioassay with those of a standard susceptible laboratory colony which has been maintained without exposure to any insecticides since 1993. Results indicated that populations from all regions have increased their resistance to the standard pyrethroid, but there is little or no resistance to spinosad and indoxacarb and reduced resistance to methamidophos. This mitigation of resistance in DBM is attributed partly to a decade-long regional adherence by the vegetable industry of, in particular, rotating spinosad with indoxacarb in two windows per year rotation strategy. The original insecticide resistance management rotation strategy has been updated to incorporate chlorantraniliprole registered as a foliar spray, and recently a mixture of chlorantraniliprole and thiamethoxam as a seedling drench. Meanwhile, the Environmental Risk Management Authority (ERMA) of New Zealand is reassessing a number of substances, and methamidophos and acephate are to be phased out and endosulfan has been de-registered.

In the last few years there has been a large increase in production of forage brassicas, mainly for the dairy industry, with 300,000 ha now grown for forage, vegetable and seed annually in New Zealand. This large increase provides a potential reservoir for susceptible populations of DBM, and may also be a factor in mitigating resistance. With the release of 'Bt brassicas' now unlikely in New Zealand for the foreseeable future, research is now refocusing on conventional pest management tools for advancing IPM, not only for vegetable brassicas, but also the transfer of some of these pest management tools for forage and seed brassicas.

Keywords
Integrated pest management, vegetable brassicas, diamondback moth, insecticide resistance, insecticide rotations

INTRODUCTION
An integrated pest management (IPM) program for vegetable brassicas in New Zealand was implemented in the late 1990s along with publication of an IPM manual (Berry et al. 2000; Walker et al. 2004). The initiation of this IPM program was prompted by the build-up of resistance in diamondback moth (DBM), *Plutella xylostella* to a standard pyrethroid, lambda-cyhalothrin. Resistance reached levels in three regions equivalent to those associated with control failures in North America (Cameron & Walker 1998). The levels of resistance recorded for the standard organophosphate, methamidophos, were also likely to cause control failures (Cameron et al. 1997). Discussion of this IPM program in a previous workshop (Walker et al. 2004) noted that the vegetable industry accepted recommendations that long-term mitigation of insecticide resistance in DBM required regular monitoring of trends in the major brassica-growing regions. These trends were monitored by comparing susceptibility levels with those of a standard susceptible laboratory colony which has been maintained at Mt. Albert Research Centre, Auckland, without exposure to any insecticides since 1993.

As well as monitoring resistance, an insecticide resistance management strategy was developed and implemented (Walker 2001; Walker et al. 2004). Rotating the use of different modes-of-action insecticides over an entire region in a "window strategy" based on calendar periods has proven to be an effective resistance management tactic (Roush 1989; Forrester et al. 1993). The recommended rotation strategy was based on options proposed for use in South Australia, but has been restricted to the limited range of registered products in New Zealand. The strategy recommended that insecticides be applied only in response to pest populations exceeding the specific action thresholds for cabbage, cauliflower and broccoli, and that Bt (*Bacillus thuringiensis*) products be applied to early crop stages. Then, when applications of insecticides were required for control of lepidopteran pests, the strategy relied on the use of spinosad (Success™ Naturalyte™) in the first part of the season from September to late January (the early 'window'), at which time growers should stop using

spinosad and change to using indoxacarb (Steward®) from the beginning of February onwards (Berry et al. 2000; Walker 2001, see also Table 1). These dates divide the year equally into the same number of DBM generations, based on heat unit accumulations. Subsequently, fipronil (Ascend®) has been registered and was placed in the late window as an alternative to indoxacarb (Walker 2001; Walker et al. 2004). The success of this regional (and national) approach requires the participation of a high proportion of growers. An independent survey undertaken in November 2001 reported that 90% of growers surveyed were using the recommended insecticide rotation strategy and 80% were rotating spinosad and indoxacarb (Walker et al. 2004).

In the last few years, there has been a large increase in brassica production in New Zealand, particularly in Canterbury, South Island, largely from the expansion of forage brassica grown for the dairy industry, but also growing of oil seed rape (canola) for edible oils or biofuel production. As a result, there are now approximately 300,000 ha of brassicas grown annually in New Zealand. This has led to a number of new issues affecting sustainable practices, which have an impact upon the vegetable brassica IPM program, and in particular, resistance management of DBM. The main problems are 1) lack of registered products with different modes of action for resistance management for use in forage and seed brassica production systems, and 2) brassica growers in sectors such as the dairy and seed industry being largely unaware of IPM tools (such as effective biological control agents) that they may use to manage pests in these different growing systems (Walker 2009). Therefore, the vegetable brassica industry initiated three projects funded by the Ministry of Agriculture and Fisheries (MAF) Sustainable Farming Fund (SFF) along with other industry partners. The projects were 1) to advance the existing IPM program for vegetable brassicas, 2) to assess resistance levels and status of important natural enemies in the mid Canterbury region, and 3) to improve foliage insect pest management in South Island forage, vegetable and seed brassica growing systems (Anon. 2010a).

This paper reports on field surveys undertaken to assess the trends in insecticide resistance in populations of DBM in the major brassica-growing regions. The updated insecticide resistance management rotation strategy for DBM will be presented and discuss this strategy and other recent developments and future requirements for management of DBM and for IPM, not only for vegetable crops but also for forage and seed crops will be discussed.

MATERIALS AND METHODS

Insecticide resistance surveys

Field collection of DBM was undertaken in summer (January to March) at sites where vegetable brassicas are grown throughout the year, in the major growing regions in New Zealand. Collections of about 50-100 DBM larvae and pupae were shipped to Mt. Albert Research Centre for mass production of larvae. Extra efforts were undertaken to attain field collections from the same sites used in earlier surveys (Walker et al. 2004).

The collections of DBM and the standard reference culture of DBM (designated Pukekohe 1) were reared in the laboratory using methods already developed (Cameron et al. 1997). Field-collected DBM larvae were reared on cabbage plants in the laboratory at 18-25°C (16:8 L:D cycle). The susceptibility of the field populations to the standard insecticides was compared with that of the standard DBM population. This reference population was collected from Pukekohe Research Station, South Auckland, in April 1993, where cabbages had been grown without insecticides for at least four years before this collection date.

The susceptibility of this population has previously been compared with a standard susceptible DBM population (Geneva 88) collected from cabbage near Geneva, NY (Cameron et al. 1997). The New Zealand reference population was calibrated with the Geneva 88 strain and this allowed the use of the New Zealand population as a baseline for comparison of resistance. This New Zealand population (Pukekohe 1) was found to be 10 times more resistant to permethrin at the LC_{50} than the Geneva 88 population. The Pukekohe 1 laboratory population is now used as the standard for resistance assays in New Zealand and has been maintained in the laboratory without exposure to insecticides for 18 years.

The measurements of susceptibility were based on leaf dip bioassays following the technique adopted by Cameron et al. (1997) from Shelton et al. (1993a, 1993b). Cabbage (*Brassica oleracea* var. *capitata*) leaf discs of 25 mm diameter were dipped for 5 s in water solutions of formulated insecticide plus surfactant (2-3 drops in each 100 ml of final rate). The discs were then placed on stainless steel mesh racks in a fume-hood at room temperature. A small fan was then directed at the discs for about 1 h to dry.

For the test assays, six replicates of at least six concentrations were used. Discs dipped in water with surfactant were used as controls. Discs were placed in 40-ml plastic cups with unvented lids and six larvae (third instar) were placed in each cup and allowed to feed at 25°C. Mortality was assessed at 24, 48 and 72 h. Dead larvae were defined to be those that did not move when touched with a paintbrush.

The standard population was included in each experiment to minimize uncontrolled variables. Probit analysis was used to estimate the LC_{50} for each population, using log (dose) as the explanatory variable. Data from the six replicates of each dose were pooled before probit analysis. Natural mortality was very low or nil in control (non-dosed) larvae in all assays. The fit of the data to the probit model was measured by changes in deviance and the heterogeneity factor (residual deviance) was used in estimates of confidence intervals for LC_{50}. To determine the degree of resistance (the resistance ratio (RR), the LC_{50} of each population was compared with the standard New Zealand population (Pukekohe 1), and confidence intervals were estimated for this ratio (the resistance ratio

is the reciprocal of the relative potency usually estimated in insecticide assays). The index of significance for potency estimation (g), the change in deviance when separate slopes were fitted for test and standard populations, and a plot of the data on the probit scale were used to confirm the validity of the parallel line regression implicit in the calculation of a resistance ratio. A generalized linear model (McCullagh and Nelder, 1983) with log link function and Gamma error distribution, weighted by 1/g, was used to model the derived RR values for any dependence on insecticide, time trend or regional differences. GenStat (2010) was used for all statistical analyses.

The insecticide resistance management rotation strategy for vegetable brassicas

The Fresh Vegetable Product Group of Horticulture New Zealand (previously VEGFED) initiated an industry-funded project to advance the IPM program for vegetable brassicas in New Zealand. This three-year project was supported by the MAF Sustainable Farming Fund and included tasks of reassessing the status of resistance in New Zealand populations of DBM and updating the IPM manual. As part of this process, the insecticide rotation strategy needed to be updated to incorporate new insecticides. An informal DBM insecticide resistance management–working group was formed in 2009, consisting of the senior author and New Zealand-based managers of the agrochemical companies that had insecticides recently registered for use on vegetable brassicas. This group liaised with regional grower representatives and business managers of the Fresh Vegetable Product Group of Horticulture New Zealand, plus a representative of the international Insecticide Resistance Action Committee (IRAC) Diamide working group (Group 28) to advance a practical update of the rotation strategy.

RESULTS AND DISCUSSION

Insecticide resistance surveys

Estimated resistance ratios for 12-15 field populations of DBM collected from four regions relative to the standard New Zealand population, with 95% confidence intervals, are presented for lambda-cyhalothrin and methamidophos from surveys over five years in Table 2, and for spinosad and indoxacarb over eight years in Table 3. The g values in the table highlight the occasional assay that was not as precise as most, usually because the range of doses turned out not to be ideal. For example, the assay for lambda-cyhalothrin at Puni in 2004 gave a low RR value, but this may also have been influenced by the only assessment being at 24 h (Table 2).

In summary, for lambda-cyhalothrin, there was significantly more resistance in populations from all regions than the standard. There are also significantly higher RR values for populations from the South Auckland region (Table 2) than for the rest of New Zealand, while the means for other regions were not significantly different from one another. For South Auckland, the weighted predicted mean over all years of the RR to lambda-cyhalothrin was 66 (s.e. 13.9), while for other regions in New Zealand it was 7.2 (s.e. 1.3). Also, there was a significant trend at South Auckland with increasing resistance to lambda-cyhalothrin over time. The weighted predicted mean RRs to lambda-cyhalothrin increased in South Auckland from 41 in 2004 to 68 in 2006 (not tested in 2008), and in the rest of New Zealand from 4.4 in 2004 to 12.3 in 2008 (deviance ratio 8.2; df= 1,7; P=0.024).

For the other three insecticides the trend over time was not significant, but RRs varied significantly among the insecticides (deviance ratio=30.8; df=2.32; P<0.001), with indoxacarb RR significantly higher than the other two, which were similar. Also, the RRs were higher for South Auckland populations (deviance ratio=29.6; df=1.32; P<0.01) for all insecticides. Weighted predicted means for South Auckland and the rest of New Zealand were 1.8 and 1.1 for spinosad, 2.1 and 1.2 for methamidophos, and 3.6 and 2.2 for indoxacarb, respectively.

The results, particularly for lambda-cyhalothrin, indicate that resistance was also variable within regions. For example, RRs varied between two populations within the South Auckland region in 2004 from 4.0 to 36.7 (Table 2). Also, the two populations tested from Canterbury in 2008 for resistance to indoxacarb gave RRs of 2.39 and 8.08 (Table 3).

Results were also compared over time with earlier surveys undertaken between 1997 and 2000 and reported at previous workshops by Walker et al. (2004) for three of these regions, being Pukekohe (= South Auckland), Gisborne and Canterbury for lambda-cyhalothrin and methamidophos (presented in Table 2), and spinosad (Table 3). The range of RRs increased for lambda-cyhalothrin from 2.3–62.3 in the earlier surveys to 4.0–88.5 at South Auckland and from 3.5–8.5 to 7.92–14.4 in Canterbury. The highest RR for Gisborne for lambda-cyhalothrin was the same at 7.6 in both survey periods. However, the maximum RRs for methamidophos reduced in all three regions from 4.2 to 3.85, 1.7 to 1.12, and 3.6 to 1.29 for South Auckland, Gisborne and Canterbury, respectively. The maximum RR for spinosad during this survey was 2.01 for a Canterbury population, which is less than the maximum RR reported by Walker et al. (2004) of 3.57 from 15 populations tested between 1997 and 2000.

All these surveys were undertaken with populations of DBM sourced from vegetable crops where all four products are currently registered (Anon. 2010b). However, only lambda-cyhalothrin is registered for use on other brassica crops. The large increase in brassica production in the Canterbury region is mainly for the dairy industry, where the economic injury level (EIL) caused by lepidopteran pests, including DBM, is higher than for vegetable brassicas (Walker, personal observation). Therefore, these crops normally receive fewer insecticidal applications than vegetable crops,

which provide a potentially large reservoir for susceptible populations of DBM in that region. This may be a factor in mitigating resistance in that region, but the lack of alternative modes-of-action insecticides means that DBM populations in these crops are still being subjected to applications of pyrethroids and organophosphates (G.P. Walker, unpublished data). Also, DBM resistance to lambda-cyhalothrin is significantly higher in South Auckland than elsewhere and resistance to this product is increasing over time (Table 2). This problem may well be due to continued use of this product for control of other insect pests in the warmer, northern regions of New Zealand, where the noctuid species *Thysanoplusia orichalcea* and *Helicoverpa armigera* are important pests in summer and autumn crops (Walker et al. 2009). Lambda-cyhalothyrin is also used in this region to control infestations of *Thrips tabaci* (onion thrips) in leafy vegetable crops (Anon. 2010b).

In New Zealand, insect-resistant Bt brassica crops were previously being developed for control of the target pests, DBM and white butterfly, *Pieris rapae* (Christey et al. 2006; Walker et al. 2007). However, the release of these 'Bt brassicas' is now unlikely in New Zealand for the foreseeable future. Thus, research effort needs now to refocus on conventional pest management tools for advancing management of, in particular, DBM for vegetable brassicas, but also on transferring practical IPM tools for sustainable production of forage and seed brassicas.

The insecticide resistance management rotation strategy for vegetable brassicas

The insecticide resistance management rotation strategy for vegetable brassicas required updating as new products became registered or other products were de-registered. Since the 2001 version was published (Walker 2001), those products relevant to DBM have been chlorantraniliprole registered as a foliar spray (Coragen®), and recently a mixture of chlorantraniliprole and thiamethoxam as a seedling drench (Durivo™).

Chlorantraniliprole is an active ingredient in the diamide chemical sub-group belonging to the IRAC mode of action class 28 of chemicals (IRAC 2008). The IRAC International "Group 28 Working Group" recommends a Group 28-active window and a Group 28-free window to prevent the development and spread of insecticide resistance (IRAC 2008). However, it was considered by the DBM working group in New Zealand that it was preferable to place one product in each of the two windows. This would mean that there was no Group 28-free window. This local deviation of the IRAC guidelines was brought to the attention of the manufacturers of the products and discussed with a representative of the international IRAC diamide working group. The representative of the diamide working group discussed the strategy with Australian and New Zealand teams and other insecticide resistance management (IRM) experts (JT Andaloro, personal communication) and the overall consensus was that it was a good IRM strategy to recommend. It was considered that separating the two products out in windows made sense since it prevented the possibility of spraying Durivo™-treated transplants with Coragen®. The ideal would be to have active and non-active Group 28 windows within both the early and late windows, but the two window strategy was retained for practical reasons, mainly the simplicity of use for growers.

An updated IPM program for vegetable brassicas was published in 2009 (Walker et al. 2009) and contains an updated version of the rotation strategy, including Coragen® placed in the early window. This strategy was again updated in June 2010 to include Durivo™ in the late window (Table 1). Results from an informal survey undertaken by the senior author of the major growers of vegetable brassicas and relevant agrochemical company representatives indicated that approximately 80% of the vegetable brassicas grown in New Zealand are grown using this two-window rotation strategy.

Meanwhile, the Environmental Risk Management Authority (ERMA) of New Zealand is reassessing a number of substances, and methamidophos and acephate are to be phased out and endosulfan has been de-registered.

CONCLUSION

Considering the results of these and earlier published resistance surveys, it could be concluded that pyrethroids are unlikely to be effective in controlling field populations of DBM throughout the country and that all brassica industries need to be made aware of this situation. This conclusion is reinforced when you consider that the New Zealand standard population (Pukekohe 1) was reported to be 10 times more resistant to permethrin at the LC_{50} than the Geneva 88 population (Cameron et al. 1997). Results for methamidophos indicate that there is reduced resistance to this product in the main brassica-growing regions. The higher RRs for indoxacarb in populations from South Auckland and Canterbury indicate that care is required to prevent escalation of this minor resistance in the future.

The mitigation of insecticide resistance in DBM in New Zealand may be partly attributed to the rotation of spinosad and indoxacarb over the last decade and the vegetable industry 'buy-in' of this strategy. It may also be successful because of the simplicity of the rotation strategy. The latest updated strategy has been maintained as a simple two-window strategy even though this does not accommodate the IRAC recommendation of a Group 28-free window. A future challenge will be to maintain a simple strategy that is practical for growers as new products become registered in New Zealand.

With the release of transgenic 'Bt brassicas' now unlikely in New Zealand for the foreseeable future, practical and cost-effective pest management tools such as conventional breeding for resistance, forecasting tools for predicting damaging flights of DBM (as reported elsewhere in this proceedings, (M. Walker et al. in press),

developing thresholds for new brassicas, plus the registration of new 'selective' insecticides are required.

The challenge of improving vegetable IPM and coordinating strategies with those used in other brassica crops requires that insecticide use is optimized so that the spectre of insecticide resistance is not reactivated. If DBM populations do move between crops, the positive and negative impacts of this on potential resistance should be assessed and existing techniques used to adapt thresholds, adjust timing of windows and placement of insecticides in a rotation strategy. The use of a rotation strategy has been supported internationally and its ongoing adaptation to New Zealand conditions is a cornerstone for avoiding resistance and maintaining the activity of valuable insecticides that are so important for sustainable brassica production.

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Table 1. Insecticide resistance management rotation strategy for diamondback moth (updated July 2010)

Early Window September –late January	**Late Window** February- August
Apply insecticides only in response to scouting thresholds	
Bacillus thuringiensis (Bt[1])	
spinosad (Success™ Naturalyte™)	indoxacarb (Steward®)
chlorantraniliprole (Coragen®)[2]	fipronil (Ascend®)
	chlorantraniliprole & thiamethoxam (Durivo™)[2]
	synthetic pyrethroids
organophosphates	
pirimicarb, pymetrozine (aphids)	

[1] Apply Bt to small larvae on small plants

[2] Coragen and Durivo contain the same active ingredient (chlorantraniliprole). If Durivo is used (as a transplant drench), it should not be followed with Coragen on that crop.

Table 2: Lambda-cyhalothrin (Karate®) and methamidophos (Tamaron®) LC_{50} values, their 95% confidence limits, resistance ratios (RR) and assay heterogeneity (g) for field populations of *Plutella xylostella* relative to the standard New Zealand population (Pukekohe 1), assessed at 48 hours.

			lambda-cyhalothrin				methamidophos			
Collected	Region	Population	LC_{50}	95% CI	RR	g	LC_{50}	95% CI	RR	g
2004	Sth Auckland	Puni†	0.002	0.000 – 0.009	4.01*	0.59	0.048	0.03 – 0.08	2.12*	0.08
2004	Sth Auckland	Tuakau	0.032	0.019 – 0.053	36.7***	0.08		–		
2004	Gisborne	Makauri	0.003	0.002 – 0.005	7.60***	0.14		–		
2004	Wellington	Carterton	0.003	0.002 – 0.006	4.12**	0.12	0.027	0.02 – 0.05	0.88	0.17
2004	Canterbury	Lincoln	0.002	0.00 – 0.008	5.28	0.42	0.041	0.03 – 0.06	1.33	0.06
2005	Wellington	Levin	0.002	0.001 – 0.004	3.64**	0.08	0.035	0.02 – 0.05	0.98	0.09
2006	Sth Auckland	Tuakau		–			0.108	0.08 – 0.14	1.73*	0.05
2006	Sth Auckland	Pukekawa	0.092	0.053 – 0.161	88.5***	0.06	0.232	0.18 – 0.30	3.85***	0.06
2006	Sth Auckland	Pukekohe‡	0.058	0.034 – 0.100	59.4***	0.06	0.075	0.06 – 0.10	1.23	0.07
2006	Gisborne	Makauri		–			0.047	0.03 – 0.07	1.12	0.09
2008	Canterbury	Chertsey	0.006	0.004 – 0.009	14.4***	0.04	0.042	0.03 – 0.06	1.29	0.04
2008	Canterbury	Southbridge	0.011	0.006 – 0.018	7.92***	0.09	0.038	0.03 – 0.05	1.19	0.04

† assessed after 24 h

*, **, *** significantly different from the standard population at the 5%, 1% and 0.1% significance levels

‡ Pukekohe Research Station

Table 3: Spinosad (Success®) and indoxacarb (Steward®) LC_{50} values, their 95% confidence limits, resistance ratios (RR) and assay heterogeneity (g) for field populations of *Plutella xylostella* relative to the standard New Zealand population (Pukekohe 1), assessed at 72 hours.

			spinosad				indoxacarb			
Collected	Region	Population	LC_{50}	95% CI	RR	g	LC_{50}	95% CI	RR	g
2001	Sth Auckland	Puni	0.10	0.08 – 0.11	1.21	0.03	1.76	1.30 – 2.37	4.83	0.05
2001	Sth Auckland	Tuakau	0.30	0.22 – 0.40	1.95	0.07	0.91	0.62 – 1.34	2.36	0.09
2001	Gisborne	Makauri	0.14	0.11 – 0.18	1.41	0.07	0.36	0.23 – 0.58	2.25	0.05
2004	Sth Auckland	Puni	0.08	0.04 – 0.15	1.09	0.33	1.15	0.83 – 1.59	5.91*	0.06
2004	Sth Auckland	Tuakau		–			0.18	0.09 – 0.34	0.75	0.09
2004	Gisborne	Makauri		–			6.56	2.78 – 15.5	2.39	0.13
2004	Wellington	Carterton	0.29	0.20 – 0.41	1.82*	0.10	0.25	0.14 - 0.43	1.13	0.06
2004	Canterbury	Lincoln	0.32	0.23 – 0.44	2.01**	0.09	0.09	0.05 - 0.18	0.39*	0.08
2005	Wellington	Levin	0.10	0.08 – 0.12	1.22	0.07	0.38	0.20 – 0.72	1.03	0.17
2006	Sth Auckland	Tuakau		–			0.16	0.04 – 0.74	0.59	0.23
2006	Sth Auckland	Pukekawa	0.25	0.19 – 0.33	1.75**	0.05	4.82	2.74 – 8.50	4.76***	0.05
2006	Sth Auckland	Pukekohe‡	0.22	0.16 – 0.29	1.51*	0.05	4.55	2.50 – 8.25	4.48**	0.05
2006	Gisborne	Makauri	0.18	0.12 – 0.26	1.02	0.12		–		
2008	Canterbury	Chertsey	0.14	0.10 – 0.19	0.88	0.10	14.82	4.10 – 53.59	8.08*	0.29
2008	Canterbury	Southbridge	0.12	0.08 – 0.18	0.74	0.15	4.37	1.48 – 12.94	2.39	0.23

*, **, *** significantly different from the standard population at the 5%, 1% and 0.1% significance levels

‡ Pukekohe Research Station

Crucifer vegetable insecticide resistance management strategies and issues in Australia

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ABSTRACT

The diamondback moth (DBM), *Plutella xylostella* is internationally notorious for evolving resistance to insecticides. Historically DBM resistance problems have been severe in Australian crucifer vegetable production, particularly in Queensland. This paper outlines the insecticide resistance management (IRM) program that is currently employed in Australia to maximize the number of effective applications that can be made from the available DBM insecticides, and thereby to maximize effective pest control and long term sustainability and profitability of crucifer vegetable production. A central component of this IRM program is a national "two-window" insecticide rotation strategy. This rotation strategy includes six different mode-of-action insecticide groups registered in Australia since 1998. The chosen calendar periods provide similar market share for the insecticides in each window, and take account of the dynamics of the pest complex in each State. A national resistance screening program documents changes in susceptibility to these newer compounds, and this information assists in property and regional level resistance management. Other Australian crucifer IRM strategies, which are based upon well-established pest management principles, include natural enemy conservation, threshold-based crop monitoring and well-timed and well-calibrated spray application.

Recent results of the resistance screening program indicate that moderate shifts in tolerance to emamectin benzoate, indoxacarb and spinosad have occurred in some production areas. However use of these products has declined following the 2009-10 registration of three Group-28 diamide products. The high level of diamide usage by crucifer growers nationally, and the particular resistance risk presented by one of these diamide products formulated for seedling drench application, now presents a major ongoing challenge for DBM resistance management in Australia.

Keywords

Insecticide resistance management, diamondback moth, crucifer vegetables, window strategy

INTRODUCTION

The diamondback moth (DBM), *Plutella xylostella* is internationally notorious for evolving resistance to insecticides, particularly in intensively sprayed vegetable production regions. In Australia, routine spraying for DBM and other pests in crucifer vegetable crops has led to the development of resistance to organophosphate and synthetic pyrethroid insecticides, and to the development of tolerance to some of the newer insecticides registered since the late 1990's.

This paper provides an outline of crucifer vegetable crop production in Australia, the documented history of insecticide resistance in Australian populations of DBM, and the insecticide resistance management (IRM) program that is currently employed in the Australian vegetable industry to maximize the number of effective applications that can be made from the available DBM insecticides, and thereby to maximize the long term profitability of crucifer vegetable production.

The Australian crucifer vegetable industry

The approximately 13,000 ha (AUSVEG 2009) of crucifer vegetable production in Australia is largely concentrated around the main urban population centers in each of the five mainland States and in northern Tasmania (Figure 1).

Figure 1. The location (indicated by the circles) of the six main crucifer vegetable production regions of Australia. The five southern regions are climatically temperate, and the Queensland region is subtropical

Typically a crucifer-producing farm enterprise plants between 20 to 200+ ha of crucifer vegetables per annum. In the climatically temperate southern States, DBM is the key pest, and typically has six to nine generations per annum. The great majority of the insecticide sprays, which may range from three to eight or more applications per *Brassica oleracea* crop, are applied for DBM control. In the subtropical production region of southern Queensland (Lockyer Valley), DBM has approximately 12 generations per annum, and is one of a complex of six lepidopterans (the other pest species are *Crocidolomia pavonana*, *Helicoverpa armigera*, *H. punctigera*, *Hellula hydralis* and *Spodoptera litura*) and two sucking pests (*Bemisia tabaci* biotype B and *Thrips tabaci*) that commonly require control. The number of insecticide sprays applied to Lockyer Valley crucifer crops generally ranges between 8 and 12 per crop (D. Carey, personal communication).

Insecticide sprays are generally applied using standard hydraulic nozzles mounted on approximately 10 m width boom arms. These boom spray units are tractor-mounted, and some are fitted with ducted air-assistance. Spray volumes range from 500 (transplant) to 1000 (maturity) L ha^{-1}. Some growers are starting to use fan-assisted, multiple spray heads and lesser spray volumes with excellent success (G. Furness, personal communication).

History of insecticide resistance in populations of DBM in Australian vegetable crops

From the end of WWII to the late 1970's, DDT and a range of cyclodiene, organophosphate and carbamate insecticides were used for pest control in Australian crucifer vegetable crops (Baker 1994; Endersby and Ridland 1994; Heisswolf and Hargreaves 1994). During this period, there were no documented studies of insecticidal tolerance or resistance in Australian populations of DBM. Following the registration of permethrin for the control of DBM and several other lepidopteran pests in Australian crucifer vegetable crops in 1978, a further three synthetic pyrethoids (es-fenvalerate, deltamethrin and alpha-cypermethrin) were registered in the early 1980's.

Synthetic pyrethroid resistance was first identified in DBM in populations in the Lockyer Valley of Queensland in the mid 1980's (Wilcox 1986; Altmann 1988), approximately seven years after their initial registration. Widespread spray failures and crop losses occurred in the Lockyer Valley during 1985 to 1987, which led to the development and implementation of an insecticide resistance management (IRM) strategy in 1988 (Deuter 1989). The strategy was based on the rotation of four insecticide groups- synthetic pyrethroids, carbamates, organophosphates and cyclodienes (Deuter 1989), and was subsequently broadened to include complementary IPM practices such as summer crop production break, improved spray application, pest scouting and the use of *Bacillus thuringiensis* (*Bt*) products (Heisswolf 1992).

In southern Australia, reports of DBM control failures first emerged in 1990, and were confirmed to be related to resistance to synthetic pyrethroid and organophosphate insecticides (Baker 1994; Baker and Kovaliski 1999). Further reports of DBM control failures followed in Victoria and New South Wales in 1993-94 and in Western Australia and Tasmania in 1995. Endersby and Ridland (1997) confirmed moderate to high level resistance to permethrin in strains of DBM collected from crucifer vegetable crops in each of Victoria, New South Wales, Western Australia and Tasmania. Despite the documented resistance, the use of organophosphate and synthetic pyrethroid insecticides for the control of DBM in southern Australian crucifer vegetable crops continued. The reason for this was that between 1978 and 1997-98, no new class of insecticide was registered in Australian crucifer vegetables, and the only available insecticidal alternatives were *Bt* products. Hence control was achieved by the more frequent use of the available synthetic insecticides supplemented with the use of the *Bt* products. The broad resistance to the classes of registered synthetic insecticides limited the prospects for effective resistance management.

The development of a national IRM strategy for DMB in Australian crucifer vegetables

A new opportunity to introduce a more effective platform of IRM strategies against DBM in Australian crucifer vegetables emerged in 1996-97. For the first time, a national DBM IPM program principally involving public sector research and extension workers in each State was established. A second key stimulus was the impending registration in quick succession of four highly effective insecticides- fipronil, chlorfenapyr, emamectin benzoate and spinosad, in new chemical groups that showed no apparent cross-resistance to one another. This presented a unique opportunity for a resistance management strategy to be developed based upon the rotation of these new insecticides. Thus in August 1996, state and university researchers and pesticide company representatives began formal discussions on the development of an Australia-wide IRM rotation strategy. Over the following year, they negotiated the framework and detail of the rotation strategy.

From a purely resistance management perspective, the best strategy would have been to have four windows, one for each of the new products. In theory, this would increase the number of effective applications that could be obtained from each insecticide compared to an *ad hoc* mosaic approach (Roush 1989; Immaraju et al. 1990; Roush 1993). However, given the staggered times over which the products were being registered, difficulties in dividing the year into even quarters in terms of number of spray applications and market-share, and excessive complications to the message that could be delivered to pesticide resellers and vegetable growers, a simpler two window strategy was devised.

From considerations of pesticide use patterns and pest population pressure, the year was divided into two relatively equal periods. However, in recognition of the regional/State differences in the crucifer pest complex and timing of the peak periods of DBM pressure and consequent pesticide use patterns, three different regional versions of the strategy- 1. Queensland; 2. Western Australia and 3. South-eastern Australia (South Australia, Victoria, Tasmania and New South Wales) - each with differing calendar dates for the two window periods, were devised.

With respect to the older synthetic insecticides, a review of the literature on resistance mechanisms and Australian data on cross-resistance in DBM suggested that sufficient levels of cross resistance between carbamates, organophosphates and synthetic pyrethroids would likely limit any potential windowing benefit. Further, it was anticipated that the use of these older chemistries was likely to sharply decline in favor of the

new products. For these reasons, these older chemistries were not windowed.

Because of their selectivity, leaving *Bt* products available year round was considered preferable to restricting them to one window. Hence the use of *Bt* in the early phase of crop development (*e.g.*, 6-10 leaves), when some damage can be tolerated and spray coverage is generally good, is promoted year round to help colonizing natural enemies to establish and to reduce the selection pressure on other insecticides.

Once the "two-window" DBM IRM strategy was agreed upon in later 1997, it was actively promoted in an IRM awareness campaign run in 1998. Glossy color A4 flyers of the window strategy were circulated to all crucifer growers, crop scouts, consultants, chemical resellers and chemical company field personnel.

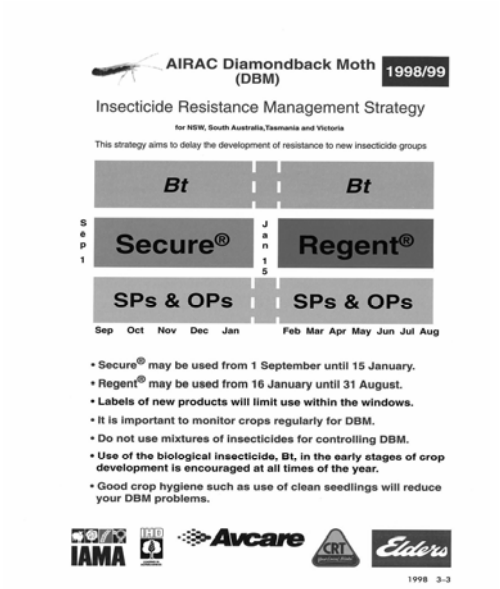

Figure 2. Version I (1998-99) of the Australian "two-window" DBM IRM rotation strategy for the south-eastern Australian geographic region

The first version (Figure 2) of the strategy windowed fipronil (Regent®) and chlorfenapyr (Secure®), which were the first of the new products to obtain Australian registration. In 1999-2000, spinosad (Success™) and emamectin benzoate (Proclaim®) were registered and added to the strategy (version II), and in 2001-02, the third version was released with the registration of indoxacarb (Avatar®). In 2009-10, the strategy was again updated (version IV, Figure 3) to include the newly registered Group-28 chemistries flubendiamide (Belt®) and chlorantraniliprole (Coragen®). All three of the regional versions of the strategy can be viewed at the following web address:

http://www.sardi.sa.gov.au/pestsdiseases/horticulture/horticultural_pests/diamondback_moth/insecticide_resistance_management

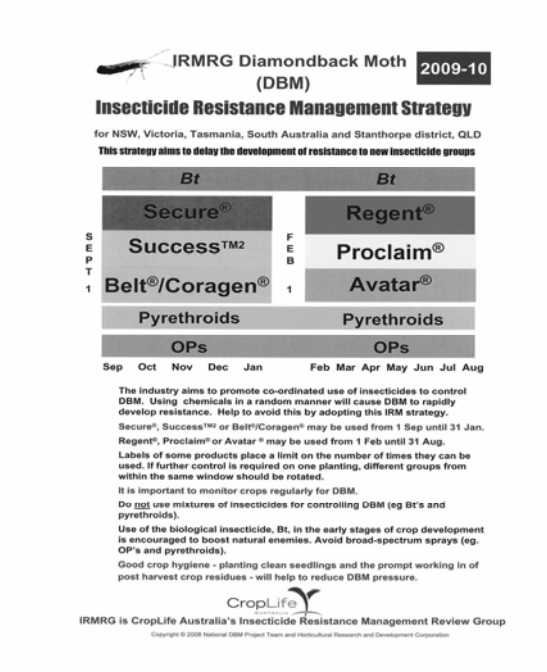

Figure 3. Version IV (2009-10) of the Australian "two-window" DBM IRM rotation strategy for the south-eastern Australian geographic region

To assist in educating growers and other industry personnel about the different insecticide classes, the opposing face of the "two-window" IRM strategy flyer contains the IRAC chemical groups and product names information.

Grower compliance with the "two-window" IRM strategy is only moderate. In a 2006 national survey of crucifer vegetable growers, 60% of respondents knew of the "two-window" DBM IRM strategy, but only 39% said that they practiced the strategy (Baker and Maratos 2007). A common reason given by growers for not using the strategy was that DBM was not considered a major pest on their property. This is concerning because if growers are still spraying frequently, albeit against low density DBM infestations, selection for resistance will continue to occur.

This level of non-compliance with the "two-window strategy" means that a mosaic of treatment patterns is often occurring in neighboring crops. Clearly if gene flows are occurring between these crops, this will result in simultaneous selection with several pesticides at the same time. However, if neighboring crucifer properties are at least one to two kilometers apart and growing crucifer crops all year round, as is often the case in southern Australia, the gene flow between neighboring properties may be quite low. For example, in release-recapture studies using laboratory-reared and fluorescent-marked DBM adults, Mo et al. (2003) estimated that 99.90% of the released moths in commercial cauliflower and broccoli crops in South Australia were expected to remain within 113 to 300 m of the release point. Hence growers that do adhere to the "two-window" strategy

may still benefit from doing so, irrespective of their neighbours' non-compliance.

In an endeavour to increase the industry awareness of the threat of DBM resistance and of good IRM practices, including the national "two-window" strategy, a DVD titled "The Reality of Resistance: Insecticide resistance development and management in Brassica vegetables" was produced in 2007 and distributed to all chemical resellers in vegetable production areas nationally.

Other key IRM strategies

In addition to the "two-window" rotation strategy, a number of other practices, which are based upon well-established pest management principles, are actively promoted for the resistance management benefits they provide. These practices aim to preserve susceptible individuals by reducing spray frequency, and include natural enemy conservation, threshold-based crop monitoring, crop breaks and well-timed and well-calibrated spray application.

Natural enemy conservation

Growers are encouraged to choose selective chemistries to minimize the disruptive effect of the spray program on the natural enemy complex. To assist growers a glossy colour "Impact of Insecticides on Natural Enemies Found in Brassica Vegetables" chart has been produced, hard copies distributed nationally, and also made available at the following web address: http://www.sardi.sa.gov.au/pestsdiseases/research_projects2/research_projects/diamondback_moth/impact_of_insecticides_chart.

Spray decision making

The national DBM IPM program has actively promoted the uptake of spray decision making based on crop scouting and the use of economic thresholds. The use of crop scout consultants, particularly those who encourage growers to implement an IPM strategy (such as IPM Technologies Pty. Ltd.) has been promoted. This program has also developed an electronic sampling plan, which assesses the need to spray based on the number of plants infested with DBM larvae, the crop type, market destination, the stage of crop development and parasitism levels (Hamilton et al. 2004). A sub-component of the sampling plan, a DBM rate of development model which requires daily max/min temperatures, assists with optimizing spray timing. Accessible at the web address: http://new.dpi.vic.gov.au/agriculture/pests-diseases-and-weeds/pest-insects/ag0512-diamondback-moth/sampling-plan, this plan interfaces with other tools developed by the national DBM program. For example, if the plan recommends a spray, then there is a direct link to the "two-window" IRM strategy so that only those chemicals available at that time of the year are recommended. Next, of the chemicals recommended, there is a link to the *Beneficial Insect Toxicity Chart*. To help conserve beneficial insects, the grower can then choose insecticides that will be less disruptive to the types of beneficials that were present during crop monitoring.

Crop break and hygiene

A crop break is promoted in Queensland from November to January inclusive, as this summer period is sub-optimal for the production of quality crucifers, particularly cauliflowers and cabbages. Good crop hygiene is promoted nationally, particularly the planting of clean seedlings and the prompt working in of post harvest crop residues. These strategies are designed to reduce DBM population densities on farm and hence to reduce spraying frequency.

Resistance screening program

A national DBM resistance screening program was established in 1999. The program involves testing of field populations of DBM from the major crucifer production regions with a variety of newer (post 1997 registration) and long-established insecticides to detect substantial change in susceptibility and to confirm resistance in the event of field control failure. Tests conducted between 2000-03 using the leaf dip method (Tabashnik and Cushing 1987) revealed that moderate levels of resistance to synthetic pyrethroids (resistance ratios, RR, of field populations compared with susceptible Waite laboratory population up to 18 fold), and no evidence of tolerance to either chlorfenapyr (RR of 0.42-2.04), emamectin benzoate (RR of 0.72-4.43), spinosad (RR of 0.68-3.40) or indoxacarb (RR of 0.83-2.48), and, with the exception of one Queensland field population (RR of 11.03), no evidence of fipronil tolerance (RR of 0.99-2.37) was observed (Endersby et al. 2004).

A further round of bioassays were conducted between 2006-10 with the three most frequently used newer DBM insecticides, *viz.*, emamectin benzoate, indoxacarb and spinosad, and *Bt* subsp. *kurstaki*. These were tested against fourteen, six, eight and two field strains, respectively. The test insecticide doses were applied by Potter tower to third instar DBM larvae on 90 mm diameter cabbage leaf discs. In 18 of the 30 bioassays, nil or very low insecticide tolerance (RR<5) was recorded (Table 1). In the case of emamectin benzoate, seven of the fourteen bioassays indicated low to moderate tolerance (5<RR<15), with the highest RR being 12.4 for a 2007 Queensland strain. With indoxacarb, four of the six bioassays indicated low to moderate tolerance, with a maximum RR of 13.3 recorded for a 2010 New South Wales strain. With spinosad, only one of the eight strains had a RR exceeding 5.0 (a 2007 Queensland strain with RR of 6.0), and with the two *Bt* subsp. *kurstaki* bioassays, intolerance was evident. Over the four years that the bioassays were conducted, there was little indication of an increase in tolerance to the tested products.

The small sample size of this 2006-10 survey does not preclude that higher tolerance levels to these insecticides may occur in some populations of DBM in Australian crucifer vegetable crops. However thus far, there have been no reports of control failures with these products nationally. Further, the tested strains were chosen from

Table 1. LC_{50} estimates for 14 DBM field strains collected from commercial crucifer vegetable crops in Queensland (QLD), New South Wales (NSW) and South Australia (SA), 2006-10

Field Strain	LC_{50} †	95% CI	RR
Emamectin benzoate:			
QLD†† 2006	0.260	0.167-0.392	6.1
QLD 2007a	0.083	0.047-0.126	1.9
QLD 2007b	0.139	0.110-0.173	3.2
QLD 2007c	0.216	0.163-0.286	5.1
QLD 2007d	0.069	0.057-0.083	1.6
QLD 2007e	0.528	0.427-0.660	12.4
QLD 2007f	0.229	0.123-0.383	5.4
QLD 2007g	0.251	0.202-0.308	5.8
QLD 2008a	0.150	0.110-0.220	5.4
QLD 2008b	0.128	0.084-0.176	4.6
QLD 2008c	0.251	0.185-0.326	9.0
QLD 2009a	0.176	0.136-0.224	4.1
NSW 2010a	0.202	0.150-0.268	4.7
NSW 2010b	0.092	0.072-0.114	2.1
Indoxacarb:			
QLD 2007a	30.9	24.9-38.4	8.5
QLD 2007c	21.3	16.2-27.3	5.9
QLD 2007h	21.15	16.08-26.97	5.9
QLD 2009a	12.9	9.3-16.5	3.6
NSW 2010a	9.18	7.26-11.13	2.6
NSW 2010b	48.0	29.7-63.0	13.3
Spinosad:			
SA 2006	1.032	0.828-1.272	3.4
QLD 2007a	1.404	1.152-1.716	4.7
QLD 2007g	1.80	1.32-2.40	6.0
QLD 2007h	1.26	1.02-1.55	4.2
QLD 2008a	0.972	0.792-1.188	3.1
QLD 2008d	0.780	0.612-0.984	2.5
QLD 2009a	0.324	0.252-0.408	1.1
NSW 2010a	0.816	0.660-0.996	2.7
***Bacillus thuringiensis* subsp. *kurstaki*:**			
QLD 2009a	90780	68839-111080	0.7
NSW 2010a	143770	106790-182779	1.1

† LC_{50} values expressed as mg a.i. L^{-1} for the 3 synthetics, and international units of potency L^{-1} for the Btk.
††QLD=Lockyer Valley, QLD; NSW=Sydney Basin, NSW; SA=Adelaide Hills, SA. For a given year, the same letter indicates a strain from the same property.

high-risk districts with a history of DBM resistance to older chemistries, so the non-detection of higher levels of resistance in these strains is an encouraging result.

Management of the new group-28 diamides

In 2009, the first of the Group-28 diamide insecticides were registered in Australian crucifer vegetables as foliar spray formulations for the control of a suite of lepidopteran pests, including DBM. The chemicals were flubendiamide (registered as Belt® 480SC and Belt® 240 WG containing 480 g ai L^{-1} and 240 g ai kg^{-1}, respectively) and chlorantraniliprole (registered as Coragen® containing 200 g ai L^{-1}). They combine high insecticidal activity and selectivity on target lepidopterans, thereby providing excellent field control with minimal toxicity to beneficials and other non-target organisms, including humans. These new Group-28 foliar spray products were placed in the first window of the Australian "two-window" DBM IRM strategy in 2009. This was publicized with the national mail-out of an updated flyer in early 2010 (Figure 3).

However looking forward, there are several specific challenges to the effective management of the resistance risk to the Group-28 chemistry. Firstly, in the two years since their initial registration, the uptake of Group-28 products by Australian vegetable growers has been rapid, and their resultant market-share is already significant. Secondly, in 2010 a seedling drench formulation (Durivo®) containing a mixture of chlorantraniliprole (100 g ai L^{-1}) and thiamethoxam (200 g ai L^{-1}) was registered for use in this crucifer vegetable market for lepidopteran and sucking pest control. The convenience and insurance value of a prophylactic seedling drench treatment is likely to encourage many growers to treat successive plantings of crucifer seedlings. This seedling drench treatment is more persistent than a foliar application. Both these factors combine to increase the resistance selection risk.

In regard to the persistence concern associated with Durivo®, an experiment was conducted recently in an Adelaide Hills Brussels sprouts crop. Seedlings sourced from three different nurseries were treated with the registered rate of Durivo® (3 g chlorantraniliprole per 1000 seedlings). At a range of intervals after transplanting, third instar DBM larvae were placed on leaf discs form the youngest fully expanded leaf from each of the nursery sources. The Abbott's corrected mortality of these larvae remained at nearly 100% for 30 DAT, and then progressively declined from approximately 90% at 35 DAT to 20% at 57 DAT (Figure 4). Unfortunately a comparative data-set for the foliar formulation of chlorantraniliprole is not presently available.

The relatively long period of partial control observed in this South Australian study is a concern, and the total exposure period is problematic for achieving compliance with two of the key global guidelines of the IRAC Diamide Working Group (IRAC 2010). These guidelines advise to "avoid exposure of consecutive insect pest

generations" to Group 28 insecticides, and that the "total exposure period of all Group28-active windows applied through the crop cycle (from seedling to harvest) should not exceed 50% of the crop cycle". In all crucifer production regions of Australia it would be unavoidable to expose consecutive DBM generations to chlorantraniliprole in any Durivo®-treated crop; and in leafy crucifer vegetable, broccoli, cabbage and cauliflower crops treated with Durivo® the total exposure period of chlorantraniliprole will likely exceed 50% of the crop cycle.

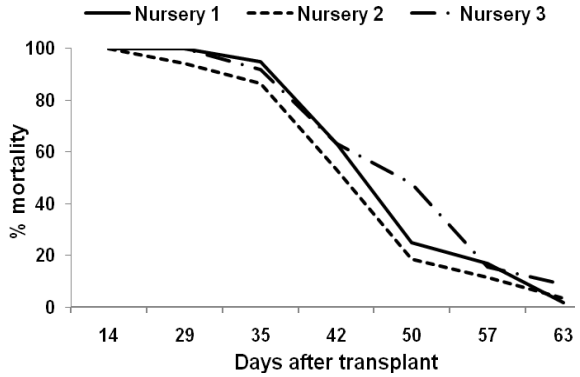

Figure 4. The Abbott's corrected percentage mortality of third instar DBM larvae after 72 hours exposure on leaf discs cut from Durivo®-treated Brussels sprouts plants at 14 to 63 days after transplant, Adelaide Hills, South Australia. The transplants were drench-treated with Durivo® (30 ml product per 1000 seedlings) and planted on 6 December 2010

A future challenge also looms with the anticipated registration of more Group 28 products, and the possibility that some of these future registrants may not wish to 'window' their products.

To provide stewardship of the Group-28 chemistry in Australia, and to help address the aforementioned challenges, an Australian Diamide Working Group has been formed with chemical company, reseller and state entomologist representatives. An initial recommendation of the Working Group to be promoted to industry is that a grower that has used Durivo® should, to help counter the risk of selection for Group 28 resistant heterozygotes, automatically rotate to another mode of action insecticide from 30-35 days after transplant and refrain from any further use of Group 28 insecticides on the crop.

CONCLUSION

Significant improvements in Australian crucifer pest management and, specifically, in the management of insecticide resistance in diamondback moth populations in this cropping system, have been achieved in the past fifteen years. The challenge to further increase grower adoption of these practices and to effectively incorporate new chemistries, particularly seedling drench formulations, into these IRM strategies is ongoing and increasing.

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Management of insecticide resistance development in diamondback moth, *Plutella xylostella* (L.)

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ABSTRACT
Diamondback moth (DBM), *Plutella xylostella* (L.), is one of the most serious pests on crucifer crops, due mainly to the development of insecticide resistance in DBM. Development of resistance to insecticides is a response of adaptation in insects to insecticide selection. Thus, the most important method to manage development of insecticide resistance is to reduce selection pressure of insecticides to insects. Based on studies on mechanisms of insecticide resistance in DBM, we considered the following strategies 1) reduce selection pressure by insecticides, and 2) control DBM without insecticides. The first strategy includes early monitoring of insecticide resistance with biochemical or molecular methods, use of insecticides that show no cross resistance or negatively correlated cross resistance, use of insecticides that show selective toxicity between DBM and natural enemies, etc. The second strategy includes use of natural enemies including insecticide resistance, cultural control methods, physical control methods, sex pheromone, etc.

Keywords
Diamondback moth, insecticide resistance, management of insecticide resistance, resistance monitoring, selective insecticide

INTRODUCTION
The diamondback moth (DBM), *Plutella xylostella* (L.) is one of the most notorious pests in the world. We can easily understand the importance of DBM from the fact that international workshops on the management of DBM and other crucifer crop pests have been held since 1985 in Taichung, Taiwan. Why has DBM become so troublesome? According to Yamamada (1977), 1) year round cultivation of crucifer crops, 2) increase of cabbage cultivation area, and 3) development of insecticide resistance of DBM were thought to be major causes for the increases of damage by DBM in Japan. If we look at the crucifer crops cultivation in Southeast Asian countries, causes of the increase of damage in crucifer crops by DBM are similar to those of Japan. High fecundity of DBM (more than 200 eggs/female) and short life span (20-30 cycles/year) were reported by Ouab et al. (1989). DBM does not show diapause. Thus, if DBM can feed on crucifer crops or weeds, it is very easy for them to develop. At this moment, there is no DBM tolerance in commercial crucifer crops (Eckerd et al. 1986, Dixon et al. 1986). The short life span of DBM in tropical countries is very convenient for DBM to develop insecticide resistance.

When we started research on insecticide resistance in DBM in 1980, there was little information on insecticide resistance in DBM in Japan. Most reports on insecticide resistance (including pyrethroids) came from Southeast Asian countries. One of our aims in conducting research on pyrethroid resistance in DBM was to obtain pyrethroid-resistant populations and clarify mechanisms of pyrethroid resistance before pyrethroids are released to the market in Japan.

We gave selection pressure to DBM with fenvalerate 16 times during 23 generations in the laboratory. In spite of long-term selection for resistance with fenvalerate, the development of resistance to fenvalerate was very slow. The highest resistance factor of LD_{50} was 8.7, which was obtained after the 12^{th} selection generation (Noppun et al. 1986). However, when fenvalearate was first introduced to the Japanese market in 1983, some studies showed that field populations of DBM in Okinawa and Kagoshima were resistant to fenvalerate in 1984 (Hama 1986). Selection of field-collected populations (Okinawa, Kagoshima and Shizuoka) of DBM with fenvalerate produced very high levels of resistance toward this compound (resistance factors at LD_{50} were from 55 to 3500) after 8 or 9 selections with fenvalerate. This indicates that DBM in Japan already possesses high gene frequencies for fenvalerate resistance after the introduction of fenvalerate to the Japanese market (Noppun et al. 1987).

METHODS TO REDUCE SELECTION PRESSURE BY INSECTICIDES

Rotational use of insecticides that do not show cross resistance
Development of insecticide resistance is a response of insects to selection pressure by insecticides. Thus, the most important method to avoid or manage the development of insecticide resistance is to reduce selection by the same insecticides, if the same mode of action of insecticides or the same degradation enzymes are involved in these insecticides (Saito et al. 1995). If we can find a combination of insecticides that shows negatively correlated cross resistance, it will be an ideal method to manage the development of insecticide resistance (Nomura et al. 1999). At this moment, such combinations of insecticides were reported only in rice green leafhopper, *Nephotettix cincticeps* Uhler.

Usually insecticide resistance levels are not stable under insecticide-free conditions. Insecticide resistant populations usually spend more resources than the susceptible one, especially under severe conditions. This may lead to the recovery of insecticide susceptibility under higher temperature conditions because of the fitness cost in the resistant population. Cases of significantly declines in resistance levels were found in field DBM populations in summer (Liu et al. 2008). If the spray interval of the same insecticide is elongated or lower dosages of insecticide are applied, there will be more chance to avoid the development of insecticide resistance.

It is important to carefully manage the use of insecticides. If a very effective insecticide is released into the market, farmers will want to apply it many times during one crop season. Now we have many kinds of insecticides to control DBM. Depending on the countries, regions and areas, we should prepare a suitable package of insecticides to control DBM. This may help reduce selection pressure of the same insecticides or same group of insecticides, and avoid the development of insecticide resistance.

Reserve natural enemies or use of insecticide resistant natural enemies

One method to reserve natural enemies is to use selective insecticides (Saito et al. 1991). Another is to use insecticide that is resistant to natural enemies. If we can protect more natural enemies, we can increase the intervals between insecticide sprays. If we can use insecticide resistant natural enemies, we can expect more natural enemies to survive after an insecticide spray. Thus, we can expect less selection pressure of insecticides (Wu et al. 2004).

Monitoring of insecticide resistance

At the early stage of the development of insecticide resistance, it is not easy to detect insecticide resistance by bioassay methods. Molecular or biochemical methods to monitor resistance gene are very effective. Based on the information, we can select suitable insecticides and avoid giving excess selection pressure to the population. These methods were designed based on the results of mechanisms of insecticide resistance. Increased degradation of insecticides (Sonoda and Tsumuki 2005, Bautista et al. 2007) and decreased sensitivity of target site (Kato et al. 1987, Kwon et al. 2004, Baek et al. 2005) are major methods to detect resistance genes. If there is no information on mechanisms of insecticide resistance, a simple bioassay method will be useful to know the susceptibility of a field population.

INTRODUCE ALTERNATE CONTROLLING METHODS

To reduce selection pressure by insecticides, the most effective method is to limit the use of insecticides as much as possible.

Sex pheromone as a mating disrupter

Sex pheromone of DBM was first observed by Chow et al. (1974). Tamaki et al. (1977) identified the contents of sex pheromone (Z-11-hexadecenal and Z-11-hexadecenyl acetate). As a mating disrupter, sex pheromone of DBM is very effective. However, to prevent the effect of immigrants, a large area of a crucifer crop field (at least few hectares) should be treated with sex pheromone. Even if crucifer crops are not cultivated in an area, it still should be treated with sex pheromone. For more effective control, crucifer crop fields should be flat and without strong winds, so that the pheromone odor can linger in the crucifer crops fields (Nemoto et al. 1992, Ohbayashi et al. 1992, Ohno et al. 1992).

Monitoring of DBM population

Conventional light traps are widely used to monitor many insect populations. Currently in Japan, sex pheromone traps and yellow sticky traps are useful tools to monitor DBM populations in the fields (Saito et al. 1990). Yellow sticky traps were first introduced to monitor field populations of DBM. However, in the fields so many DBM moths were captured on yellow sticky traps that this method also was used to control or reduce DBM moths. Pheromone traps are sensitive and species-specific; thus, even under low population conditions, more male moths can be trapped.

Physical and mechanical methods to control DBM

Meshed screen, yellow traps and yellow lights are effective tools for DBM control. If yellow sticky tape is treated with pyriproxyfen, we can expect not only mortality of DBM moths but also a decline in the next generation (Oouchi 2005).

Meshed screen can prevent DBM moths from entering into greenhouses, vinyl houses or vinyl tunnels. However, during the hot season, the temperature inside net house can rise and may affect the development of crops. Yellow lights can prevent DBM moths from entering glass or vinyl houses. Yellow lights are effective in open fields.

Relationship between plant, DBM and natural enemy

When cabbage plants are damaged by DBM, they release certain chemical substances to attract natural enemies (*Cotesia plutellae*) of insect pests. Recently, chemical substances involved in this phenomenon were identified (Shiojiri et al. 2000). Using these chemicals in the fields, we can attract more natural enemies to control DBM.

Stop cultivation of crucifer crops for certain term

One reason DBM became an important pest for crucifers was the year-round cultivation of crucifer crops (Yamada 1977). If cultivation of crucifer crops ceases for a while in a wide area, the DBM field population will decline. DBM can feed only on crucifers. Populations of crucifer weeds should be kept low for this method to reduce DBM populations.

CONCLUSION

DBM resistance is not easy to manage. However, by reducing selection pressure of insecticides in different ways, the development of insecticide resistance in DBM can be managed. In Japan, DBM is not as important a pest of crucifer crops as it was one or two decades ago. We are not sure what the major cause is for this situation. One important reason is that Japan has many registered insecticides to control DBM. Farmers can select the proper insecticide depending the season and developmental stage of crucifer crops, which may have reduced selection pressure of insecticides on DBM. This aspect of resistance could be an area for future research. Another way to reduce selection pressure of insecticides is to introduce alternate DBM control methods.

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SESSION 6

Overcoming barriers to development and implementation of IPM systems for crucifers

Impact of reduced-risk insecticides against insect pests on cabbage (*Brassica* spp.)

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ABSTRACT

Field trials were carried out on cabbage plantings in 2009 and 2010 that compared neem (Aza-Direct) and Dipel (*Bacillus thuringiensis*) with available reduced-risk insecticides. Cabbage plantings at University of Guam Agricultural Experiment Stations in Yigo and Inarajan used 6 application of Aza-Direct and Dipel on a rotational basis (current recommended practice) while the other reduced-risk treatments consisted of 6 or 8 applications of Volck Oil Spray, BotaniGard and EcoSmart or control treatment. Each treatment was assessed for pest damage and yield levels. The current recommended practice reduced the pest populations and damage, resulting in better yield compared to other available reduced-risk insecticides or control treatment. Cabbage growers were invited to attend the training program on the benefits of reduced-risk insecticides at the College of Natural and Applied Sciences, University of Guam. Two months after the training program, the attendees were surveyed for long- term gains in their knowledge of cabbage cultivation and reduced-risk insecticide practices. Forty cabbage growers from different islands attended the training program; 36 growers realized the risks associated with the toxic chemicals and established the concept of using reduced-risk insecticides in controlling the cabbage pests. However, follow up with the growers indicated that 24 of the growers actually implemented the program involving reduced-risk insecticides such as neem + DiPel in the Mariana Islands.

KEYWORDS

Reduced-risk insecticides, *Spodoptera litura*, *Liriomyza brassicae*, cabbage, Guam

INTRODUCTION

Cabbage (*Brassica* spp.) has been grown in the Marianas for many years. It is an extremely popular vegetable in Micronesia today. Marianas production of cabbage is exclusively for self-sufficiency and in some cases, fresh market. In many parts of the Marianas, cabbage crops are not under continuous cultivation, and periodic cropping systems may demonstrate different patterns of pest occurrence. Cabbage (*Brassica oleracea*) is the most frequently grown cruciferous crop in subsistence arenas in Guam and other neighboring islands. The subsistence growers' crops are considered patchy because the cabbages are grown in small and relatively widely separated plots.

Insects establish the principal pest group on cabbage in the Marianas. The greatest insect problem for growers in the region is the cutworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) and the serpentine leaf miner, *Liriomyza brassicae* Riley (Diptera: Agromyzidae) (Reddy 2011). The webworm, *Hellula undalis* Guenée (Lepidoptera, Pyralidae) occurs only at the seedling stage. The diamondback moth (*Plutella xylostella* L. (Lepidoptera: Plutellidae) occurs only when cabbage is under continuous cultivation. Traditional grower practices tend to influence the development of integrated pest management (IPM) programs adopted by these growers. Previous studies dealt with the development of IPM based on quick and easily available environmental control agents. The IPM treatments, (six or eight applications of neem and DiPel on a rotational basis) reduced pest populations and damage, resulting in a better yield than did the standard chemical or control treatment (Reddy 2011). When IPM treatments included three applications of neem plus 3 applications of DiPel (on a rotational basis in experimental fields), it again reduced the pest population and damage and produced a better yield than did the standard practice. The lower input costs of the IPM program resulted in better economic returns in both trials.

The aim of the current study is to evaluate the effect of reduced-risk insecticides against cabbage pests in comparison with a neem-based biopesticide (Aza-Direct) and DiPel, both of which are reported to be effective against many insect pests and have low environmental impact.

MATERIALS AND METHODS

Specifics of the experimental fields

The cabbage seeds of K-K Cross (American Takii Incorporated, Salinas, CA) were sown in trays (40 × 30 cm) and raised in a nursery in the greenhouse (28–30°C, 40–60% RH, 15:9 L:D h). The seedlings were grown for 35 days. Field studies were carried out at two locations of the University of Guam Agricultural Experiment Station, at Yigo (13°31.930'N, 144°52.351'E) during August–November 2009 and the second at Inarajan (13°61.963'N, 144°45.353'E) during February–May 2010. The treatment plots were 8.0 m × 8.0 m and separated from other plots by 1.5m buffer zones designed to elude spray drift or other treatment effects. Cabbage seedlings raised in the nursery were transplanted to the experimental plots, and all recommended agronomic practices were followed. At each of the two locations, 35-day-old cabbage seedlings were planted with 60 × 75 cm spacing. Three replicates of each of 11 treatments produced a total of 33 plots. Each plot consisted of 12 rows of 12 cabbage plants, for a total of 144 plants per plot. The total area of the

experimental cabbage field was 2781 m^2 (0.24 acre) at each site.

Details of reduced-risk insecticides

The 10 treatments were applied in a randomized block design, as shown in Table 1. The concentrations of treatment applications were as follows: Aza-Direct® (1.2% Azadirachtin, Gowan Company, Yuma, AZ), 10 ml/liter; DiPel® DF (Valent BioSciences, Libertyville, IL), 15 g/liter; Volck® Oil Spray (The Ortho Group, Marysville, OH), 19.5 ml/liter; BotaniGard® 22WP (Laverlam International Corporation, Butte, MT), 2.4 grams/liter; and EcoSmart® Spray, EcoSmart Technologies, Alpharetta, GA, (contains: Rosemary oil: 0.25%, Pepermint oil: 0.25%, Thyme oil: 0.25%, Clove oil: 0.25%, Other ingredients: 99.00%), whole spray. The amount of fluid sprayed per application was approximately 93.5 liters/hectare for small plants (up to 45 DAT) and 187.0 liters/hectare for larger ones (45 DAT until harvest). All the chemicals were applied with motorized backpack sprayers (Solo Brand; Forestry Suppliers, Jackson, MS). The sprayer was equipped with an adjustable, flat spray, hollow cone, and jet stream nozzle and pressure was calibrated to deliver 20 gpa (185.35 liters/ha) at 45 psi.

Table 1. Details of the treatments imposed on cabbage fields at Yigo and Inarajan on Guam

Treatment	Timing of treatment application (Days after transplanting)
Control (no applications)	
Neem, 8 applications	15, 25, 35, 45, 55, 65, 75, and 85
DiPel, 8 applications	15, 25, 35, 45, 55, 65, 75, and 85
Volck Oil Spray, 8 applications	15, 25, 35, 45, 55, 65, 75, and 85
BotaniGard, 8 applications	15, 25, 35, 45, 55, 65, 75, and 85
EcoSmart, 8 applications	15, 25, 35, 45, 55, 65, 75, and 85
Neem, 3 applications, alternating with DiPel, 3 applications	15, 30, 45, 60, 75, and 90
Volck Oil, 3 applications, alternating with BotaniGard, 3 applications	15, 30, 45, 60, 75, and 90
Volck Oil, 3 applications, alternating with EcoSmart, 3 applications	15, 30, 45, 60, 75, and 90
BotaniGard, 3 applications, alternating with EcoSmart, 3 applications	15, 30, 45, 60, 75, and 90

Damage and yield assessment

For both the 2009 and the 2010 field trails, sampling was done weekly for 16 weeks. Incidence of attack by *S. litura* was measured as the number of insects found of each stage (eggs, larvae, and pupae) and number of holes on the leaves, and as the number of larval mines by *L. brassicae* observed (Reddy 2011). Ratings of the damage they caused on 10 randomly selected plants in each treatment plot were recorded (Table 2). Each cabbage head was evaluated for head infestation (immature pest stages on head) and marketability using a standard 1-6 scale (Greene et al. 1969). At the end of the trial, the crops were harvested and the yield was recorded for each treatment. The data were averaged and expressed as the number of larvae and holes and as damage per plant and yield per hectare.

Evaluation of occurrence of natural enemies

Any occurrence of natural enemies (parasitoids and predators) of the pests was also recorded for each treatment plot during sampling for the pests. Three cabbage leaves with eggs and ten larvae of *S. litura* from each plot were collected and incubated, and emergence of parasitoids was noted for measures of parasitism. Similarly, three cabbage leaves heavily infested with *L. brassicae* were collected and examined for any parasitoid emergence. All samples were placed individually in plastic boxes with a perforated top for aeration. They were transported to the laboratory and stored at room temperature until any parasitoids emerged (Reddy 2011).

Training producers and stakeholders about reduced-risk insecticides

A list of cabbage growers is provided in Table 4. These growers were invited to attend the training program on Wednesday 16th, February, 2011 about the reduced-risk insecticides at the College of Natural and Applied Sciences, University of Guam. This training program was conducted in cooperation with the Cooperative Extension Units of the University of Guam for the benefit of the growers. This training program should inform growers of the risks associated with conventional insecticides and illustrate the benefits of ecologically sound reduced-risk insecticide practices.

Follow up with growers after a training program

Two months after the training program, the attendees were surveyed for long- term gains in their knowledge of cabbage cultivation and reduced-risk insecticide practices. The impact of the training program on reduced-risk insecticides were measured by a survey of growers who have experience in planting cabbage.

Statistical analysis

All the data were analyzed by the SAS GLIMMIX procedure in SAS version 9.2 (SAS Institute, Inc. 2009). The data for the holes and leaf mines made by larvae were analyzed with a generalized linear mixed model with Poisson distribution, and a log-link was used to test the treatment and month effects for these count data. Averages of insect population or damage (holes and

mines) on the 10 plants in each plot were used as the dependent variable. For yield data (by site), a one-way ANOVA was performed, and if the treatment effects were significant ($P < 0.05$), mean pair-wise comparisons were performed on least squares by the least-squares-difference method. If the treatment and or month effects were significant, pairwise mean comparisons were performed with the log-transformed LSMEANS.

RESULTS AND DISCUSSION

Damage and yield assessment in the experimental plots

Table 2 presents the number of larval *S. litura*, the number of holes made in leaves by those larvae, and the number of mines made by *L. brassicae* on the experimental plots. All three variables were significantly lower ($F_{9, 184} = 124.2$, $P < 0.05$) on neem + DiPel treatment plots than on other plots. The control plots suffered the greatest damage from *S. litura* and *L. brassicae*. Results from all other treatments were in-between. The marketable yields of cabbage from the neem + DiPel plots at both locations were significantly higher than those on other plots ($F_{9, 38} = 22.2$, $P < 0.05$) (Table 3). Whole, neem + DiPel treatments produced a yield about 50% higher than that of the control plots. These results agree with the previous studies (Reddy 2011) that clearly demonstrated the neem + DiPel plots were superior in reducing the pest population density compared with the other treatments or control plots. That means that the current reduced-risk recommended practice is highly valuable and effective in controlling the pests on cabbage. In the past several years, there has been substantial effort on the development and use of reduced-risk insecticides in agricultural crops (Sæthre et al. 1999). These insecticides are newer classes of compounds that pose lower health risks to humans and the environment (Maxwell and Fadamiro 2006). These new compounds are becoming increasingly important in the pest management programs on cabbage (Burkness and Hutchison 2008). Integrated pest management programs incorporating reduced-risk insecticides were developed for the control of several insect pests on different crops, for example, grapes (Jenkins and Isaacs 2007), cabbage (Burkness and Hutchison 2008), cotton (Reddy and Manjuantha 2000), and eggplant (Reddy 2001).

Follow up with growers after a training program

The training program provided knowledge to growers about the risks associated with the toxic insecticides and about benefits of the reduced-risk insecticides. The training program was attended by 40 growers from different islands; 36 growers (90% of them) understood the risks associated with the toxic chemicals and accepted the concept of using reduced-risk insecticides in controlling cabbage pests (Table 4). However, follow up with the growers indicated that 24 of the growers (60% of them) actually implemented the program involving reduced-risk insecticides such as neem + DiPel in the Mariana Islands.

CONCLUSION

The reduced-risk commercially available products (3 sprays of Aza Direct/neem + 3 sprays of DiPel), applied alternately, was effective in controlling the pest complex on cabbage in Guam.

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Table 2. Mean numbers (±SE) of larvae of *Spodoptera litura,* holes made by larvae, and mines caused by *Liriomyza brassicae* recorded per cabbage plant grown on experimental plots at the two locations on Guam

Treatment	Yigo location			Inarajan location		
	Larvae	Holes	Mines	Larvae	Holes	Mines
Control (no applications)	34.2 ± 0.4d	136.5 ± 0.6d	72.6 ± 1.2d	26.0 ± 0.4d	122.4 ± 0.4d	86.2 ± 0.8d
Neem, 8 applications	6.4 ± 0.2b	25.2 ± 0.4b	3.8 ± 1.2b	4.8 ± 0.2b	28.4 ± 0.8b	4.4 ± 0.2b
DiPel, 8 applications	4.2 ± 0.3b	18.5 ± 0.2b	3.6 ± 0.4b	6.4 ± 1.8b	26.4 ± 1.6b	6.2 ± 1.6b
Volck Oil Spray, 8 applications	14.4 ± 1.4c	48.1 ± 1.4c	12.6 ± 1.4c	13.1 ± 0.8c	46.6 ± 1.0c	12.4 ± 0.8c
BotaniGard, 8 applications	16.3 ± 0.4c	64.2 ± 0.2c	18.9 ± 0.8c	14.6 ± 1.4c	54.4 ± 0.9c	16.6 ± 1.2c
EcoSmart, 8 applications	14.8 ± 1.7c	52.6 ± 0.4c	16.7 ± 0.6c	12.9 ± 1.3c	51.7 ± 1.2c	14.8 ± 1.6c
Neem, 3 applications, alternating with DiPel, 3 applications	0.8 ± 0.2a	1.4 ± 0.2a	1.0 ± 0.1a	0.8 ± 0.2a	0.0 ± 0.0a	0.4 ± 0.1a
Volck Oil, 3 applications, alternating with BotaniGard, 3 applications	16.3 ± 1.2c	58.6 ± 1.8c	14.2 ±1.7c	18.5 ± 0.3c	49.2 ± 1.4c	12.6 ± 0.6c
Volck Oil, 3 applications, alternating with EcoSmart, 3 applications	15.4 ± 0.2c	52.2 ± 0.5c	13.5 ± 0.2c	16.5 ± 0.2c	52.7 ± 1.3c	13.5 ± 0.2c
BotaniGard, 3 applications, alternating with EcoSmart, 3 applications	14.8 ± 0.6c	54.6 ± 1.2c	16.4 ± 0.4c	14.8 ± 0.6c	48.5 ± 1.6c	15.6 ± 0.3c

Means within each column followed by the same letter are not significantly different at the $P < 0.05$ level (generalized linear mixed model using Poisson distribution followed by pair-wise mean comparisons). Each treatment was replicated three times.

Table 3. Marketable yield of cabbage from the experimental plots

Treatment	Marketable yield (tons/ha)	
	Yigo location	Inarajan location
Control (no applications)	10.3 ± 1.2a	12.4 ± 0.8a
Neem, 8 applications	29.8 ± 0.8c	30.4 ± 1.4c
DiPel, 8 applications	26.5 ± 0.4c	28.4 ± 0.6c
Volck Oil Spray, 8 applications	18.4 ± 0.2b	25.6 ± 0.3b
BotaniGard, 8 applications	20.5 ± 1.6b	24.8 ± 0.8b
EcoSmart, 8 applications	17.8 ± 1.3b	23.9 ± 0.2b
Neem, 3 applications, alternating with DiPel, 3 applications	38.4 ± 1.6d	37.2 ± 1.2d
Volck Oil, 3 applications, alternating with BotaniGard, 3 applications	19.8 ± 1.5c	21.5 ± 1.2c
Volck Oil, 3 applications, alternating with EcoSmart, 3 applications	20.8 ± 0.4c	21.2 ± 0.3c
BotaniGard, 3 applications, alternating with EcoSmart, 3 applications	22.4 ± 1.4c	21.4 ± 1.2c

Means within each column followed by different letters are significantly different at the $P < 0.05$ level (one-way ANOVA followed by LSMEANS by the least-squares-difference method). Each treatment was replicated three times.

Table 4. Follow up with growers after a training program

Subjects	Guam	Rota	Saipan	Tinian	Total
Number of growers who attended	18	8	11	3	40
Number of growers who showed interest and accepted the concept of using reduced-risk insecticides	15	8	10	3	36 (90%)
Number of growers who have implemented use of reduced-risk insecticides	9	7	6	2	24 (60%)

Introduction and establishment of *Diadegma semiclausum* in conjunction with farmer field school for diamondback moth control in intensively-sprayed crucifer production in north-eastern Thailand

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ABSTRACT
Despite heavy reliance on chemical pesticides, farmers cannot control the Diamondback Moth (DBM), *Plutella xylostella,* in intensive crucifer production areas in Thailand's north-eastern highlands. Surveys done in 2004 to document natural biological control revealed the presence of the parasitoids *Cotesia plutellae, Macromalon orientale* and *Diadromus collaris*. These, having a combined overall parasitism rate of 42.3%, were insufficient to suppress DBM populations. Since the DBM parasitoid *Diadegma semiclausum* was not found, the Thai Government proceeded in 2005 to introduce it from the Cameron Highlands in Malaysia. Whereas *D. semiclausum* established easily in organic farms in Doi Angkhang (Chiangmai highlands), its introduction for establishment in intensively-sprayed crucifer production areas in the Phetchabun highlands needed careful planning in conjunction with IPM Farmer Field School (IPM-FFS) training. In 2005, 533 parasitoid adults (57% female) were released and 100 cocoons left out in FFS fields. Patches of crucifers were planted during off-season periods to provide year-round food for DBM, the latter as host for the parasitoids. In this field site, farmers participated in two rounds of consecutive IPM-FFS. After participation and aided by high levels of biological control from parasitoids, farmers reduced pesticide use from 18-20 times to less than two times per crop. Subsequently, more parasitoid releases took place area-wide (400 ha) in joint efforts with up-scaling of IPM-FFS training to 100 farmers. Continuous surveys done until 2010 consistently confirmed the spread and establishment of the parasitoid over 800 ha with satisfactory parasitism rates (80%) in fields where farmers practice IPM. Farmers now can produce higher quality crucifers with no concern of unacceptable pesticide residues, and have gained access to more lucrative domestic and international markets. That *D. semiclausum* can readily establish area-wide in intensively-sprayed crucifers in Phetchabun clearly illustrates the importance of coupling IPM-FFS training with parasitoid releases where crops are sprayed heavily.

Keywords
Diamondback moth, *Diadegma semiclausum*, biological control, Farmer Field School, Thailand

INTRODUCTION
In Chiangmai and Phetchabun highlands in North Thailand, the diamondback moth (DBM), *Plutella xylostella*, poses a major constraint to crucifer vegetable production. Farmers are unable to effectively control DBM despite applying chemical insecticides intensively. Surveys undertaken in 2004-5 to document existing natural biological control revealed the presence of the parasitoids *Cotesia plutellae*, *Macromalon orientale* and *Diadromus collaris*. Their overall combined parasitism of 42.3% was, however, found to be too low and incapable of providing effective DBM control (Upanisakorn et al. 2011).

The surveys failed to recover the parasitoid *Diadegma semiclausum.* That it was not found in the Phetchabun highlands despite earlier efforts to introduce it is unfortunate because *D. semiclausum* is a highly sought-after DBM parasitoid with known superior performance in controlling DBM in many parts of the world (Lim 1982; Sastrosiswojo and Sastrodihardjo 1986; Ooi 1992; Talekar et al. 1992; IIBC 1996).

Since the parasitoid complex of *C. plutellae, M. orientale* and *D. collaris* now present in both the Chiangmai and Phetchabun highlands can provide only partial control of DBM, the latter will undoubtedly continue to pose a serious problem to crucifer production there. And the farmers would persist to rely heavily on chemical control to deal with the problem. As such, it would be desirable that another effort to introduce *D. semiclausum* to supplement the existing parasitoid complex for DBM control in the highlands be undertaken. Only when such effort is successfully achieved can crucifer farming in the highlands fall in line with acceptable good agricultural practice to produce healthy and safe vegetables.

Guided by such objective, the Thai Government in 2005 proceeded with introduction of *D. semiclausum* brought in from the Cameron Highlands in Malaysia with assistance from CABI and the Malaysian Agricultural Research and Development Institute (MARDI). Following clearance after necessary quarantine requirements in the Department of Agriculture (DOA), the parasitoid was mass-reared and subsequently released; initially in two specially selected locations, one in the Royal Project organic farms in Doi Angkhang (Chiangmai highlands) and another in intensively-sprayed crucifer production fields in Thap Boek (Phetchabun highlands). Whereas the parasitoid established easily in organic farms in Doi Angkhang (Winotai et al. 2009), its introduction and establishment in intensively-sprayed crucifers in Thap Boek posed greater challenges and needed careful planning in conjunction with training of farmers in IPM Farmer Field School (IPM-FFS). How it was undertaken in Thap Boek is presented in this paper.

MATERIALS AND METHODS

In attempting to introduce *D. semiclausum* into Phetchabun highlands, it was considered crucial to first ensure the environment is conducive for establishment of the parasitoid. In particular, the site for initial release of the parasitoid should be in areas where the altitude is sufficiently high so that the temperature most of the time does not exceed 25^{o}C. This is vital because beyond this temperature, *D. semiclausum* is unable to reproduce satisfactorily and its population could quickly crash (Talekar et. al. 1992; Talekar and Lin 2002). The location for release should also have available abundant crucifers to support a continuous presence of DBM host population to sustain the parasitoid. With these requirements in mind, the location Thap Boek at an altitude of 1,565 meters, with year-round favourable weather conditions, was selected for initial release of *D. semiclausum* in the Phetchabun highlands. Typically, as for the period 7 Dec 2005 to 15 Dec 2006, it has an average temperature of 21.8°C (max 29.6°C, min 16°C, mostly between 18-23°C) and average humidity of 68.4% (max 93.9%, min 45.8%, mostly between 64-75%). Maximum temperature occurrence is rare and usually only for a very brief period, hence of no risk to *D. semiclausum*.

Another critical factor given careful consideration is that *D. semiclausum* is highly susceptible to many chemical insecticides. As such, no chemical spraying was done so that the parasitoid could survive and freely multiply. Where intervention is needed to prevent excessive DBM build-up, biological-based insecticides that are selectively safer to the parasitoid would instead be used, such as Bt or neem products. This is crucial as failure to do so could easily jeopardise the chance of establishing the parasitoid. To ensure that this prerequisite is never compromised, only farmers who have been adequately trained in IPM through the hands-on participatory and discovery-based learning process in FFS were allowed to take on the parasitoid release activities in their fields.

The efforts at releasing *D. semiclausum* in the Phetchabun highlands were carried out in two stages, as follows:

Stage 1: The parasitoids were released in a specific cabbage crop (Field 1 of FFS-trained farmer Mr. Kriangkrai Ngaopun) in conjunction with an on-going FFS at the farm. The field was planted in mid-January 2006 and harvested in April/May with harvesting completed at the end of May. A second planting began in mid-June with harvesting starting in October and continuing until the end of November. The two crops in succession were to allow ample time for the released parasitoids to produce several generations of progenies if they could survive the conditions. To ensure a continuous supply of cabbage plants at different stages and DBM hosts to sustain the parasitoids (especially when the crop stubbles of the first crop were cleared to make way for the second planting), four patches of cabbage were also planted in a staggered fashion in the vicinity just outside the main crop.

In total, only 633 parasitoids were released on two separate occasions into the first planting (Table 1). Adults were freed from a cage containing them at separate points in the field. Cocoons were kept in a box protected from the weather in the field until adults subsequently emerged to escape into the field.

Table 1. Number (No.) and stage of *Diadegma semiclausum* released in conjunction with FFS on the farm of Mr. Kriangkrai Ngaopun in Thap Boek, Phatchabun

Date	Total (No.)	No. and Stage
Nov 2005		
12	472	• 372 Adults (155 males; 217 females) • 100 Cocoons
30	161	• 161 Adults (75 males; 86 females)

About one month after the first release, farmers participating in the FFS (with help from trainer facilitators) began field monitoring for the parasitoids. This involved observing for parasitoid cocoons on plants and collecting DBM larvae to rear out the parasitoid. They were done over the first crop, including the stubbles after the harvest. During and after completion of FFS in the second planting, the farm owner continued to make periodic checks for presence of *D. semiclausum* in his cabbage crops that were cultivated two times in a year.

Stage 2: Parasitoids were released in seven fields of FFS-trained farmers spread over different locations (Table 2). This area-wide effort was undertaken jointly with the up-scaling of IPM-FFS training of farmers, an effort which involved 100 crucifer farmers. The primary target of the area-wide parasitoid releases was to expand the establishment of *D. semiclausum* over as wide an area as possible in the Phetchabun highlands.

Table 2. Area-wide releases of *Diadegma semiclausum* over different locations of Phetchabun highlands.

Date	Adults released (No.)	Sex		Locations (farms of FFS farmers)
2007		Male	Female	
22 Jan	1,300	710	590	Mr.Tirawat Saewha
26 Mar	1,200	620	580	Mr.Kriangkrai Ngaopun (Field 2)
16 Apr	900	505	395	Mr.Kriangkrai Ngaopun (Field 3)
12 Jul	60	30	30	Farm at curve road entering Thap Boek
18 Jul	20	10	10	Heaven hill
24 Aug	20	10	10	Mr.Ya Saeweu
25 Oct	100	50	50	Mr.Suriya Saelhor

RESULTS AND DISCUSSION

After about five weeks from the first release of *D. semiclausum*, both parasitoid cocoons and parasitized DBM larvae could be found in the field indicating the parasitoid was able to quickly find DBM hosts in the field to parasitize them. The level of parasitization rose rapidly from 1.3% initially in early December 2005 to 27.3% in mid-March 2006, over quite a short period of only three-and-half months (Table 3). Thereafter, the population of DBM larvae in the field was very low. Despite thorough searching, no DBM larvae could be found. On the other hand, still a few cocoons of *D. semiclausum* were recovered. However, by 17 April 2006 only one last single cocoon was found. Thereafter, until 6 June, neither *D. semiclausum* cocoon nor any DBM larva could be found.

Notwithstanding the concern and without further parasitoid introductions, cocoons of *D. semiclausum* were detected again in the second planting that followed about a month later. It was observed to be increasingly common, as well as for every subsequent crop grown at the site. Continuous surveys done until now have consistently revealed its presence and confirmed its field establishment to provide satisfactory parasitism rates of around 80%.

Subsequently in Stage 2 where more area-wide parasitoid releases were done with up-scaling IPM-FFS training of farmers (initially over 400 ha in joint efforts with 100 crucifer farmers), the undertakings have resulted in the establishment of *D. semiclausum* in all the 14 survey sites that were undertaken at the beginning of 2009. With

Table 3. Field monitoring for cocoons of *Diadegma semiclausum* and parasitization of field-collected DBM larvae.

Date	Cocoons* (No.)	DBM larvae				Remarks
		Total No. Collected	No. Survived	Parasitized**		
				No.	%	
2005						
7 Dec	0	164	156	2	1.3	Crop harvested; monitoring done on stubbles.
15 Dec	2	108	98	0	0	
2006						
11 Jan	0	42	37	0	0	Plough field; plant new crop.
22 Jan	0	152	137	4	2.9	
7 Feb	0	80	71	0	0	Planted crop in the field.
28 Feb	0	0	-	-	-	
9 Mar	0	42	31	2	6.5	
19 Mar	4	14	11	3	27.3	
25 Mar	1	0	-	-	-	
30 Mar	0	0	-	-	-	
10 Apr	2	0	-	-	-	
17 Apr	1	0	-	-	-	Crop harvested in stages.
2 May	0	0	-	-	-	
17 May	0	0	-	-	-	
24 May	0	0	-	-	-	
30 May	0	0	-	-	-	
6 Jun	0	0	-	-	-	Plough field to prepare new planting.

* *D. semiclausum* cocoons from 30 plants examined on day of monitoring.
**DBM larvae with adult *D. semiclausum* emerging.

establishment rapidly achieved by *D. semiclausum* in all these locations, the area-wide releases were further expanded to cover many more sites. Continuous surveys done up to 2010 consistently confirmed the establishment and spread of the parasitoids area-wide, having expanded to cover about 800 ha and ever present in all the 21 sites that were surveyed (Figure 1). Adult emergence of *D. semiclausum* from cocoons was generally high, averaging 91.3%. Moreover, satisfactory parasitism rates of 80% or more in fields where farmers are practicing IPM can now be commonly achieved. This is primarily because the farmers, after participation in IPM-FFS and having learnt the beneficial role of *D. semiclausum*, are actively making efforts to conserve them. With help from the parasitoids, farmers have cut down chemical pesticide use from 18-20 times to a maximum of only two times per cropping cycle. They also are now able to produce higher quality crucifers with no concern of

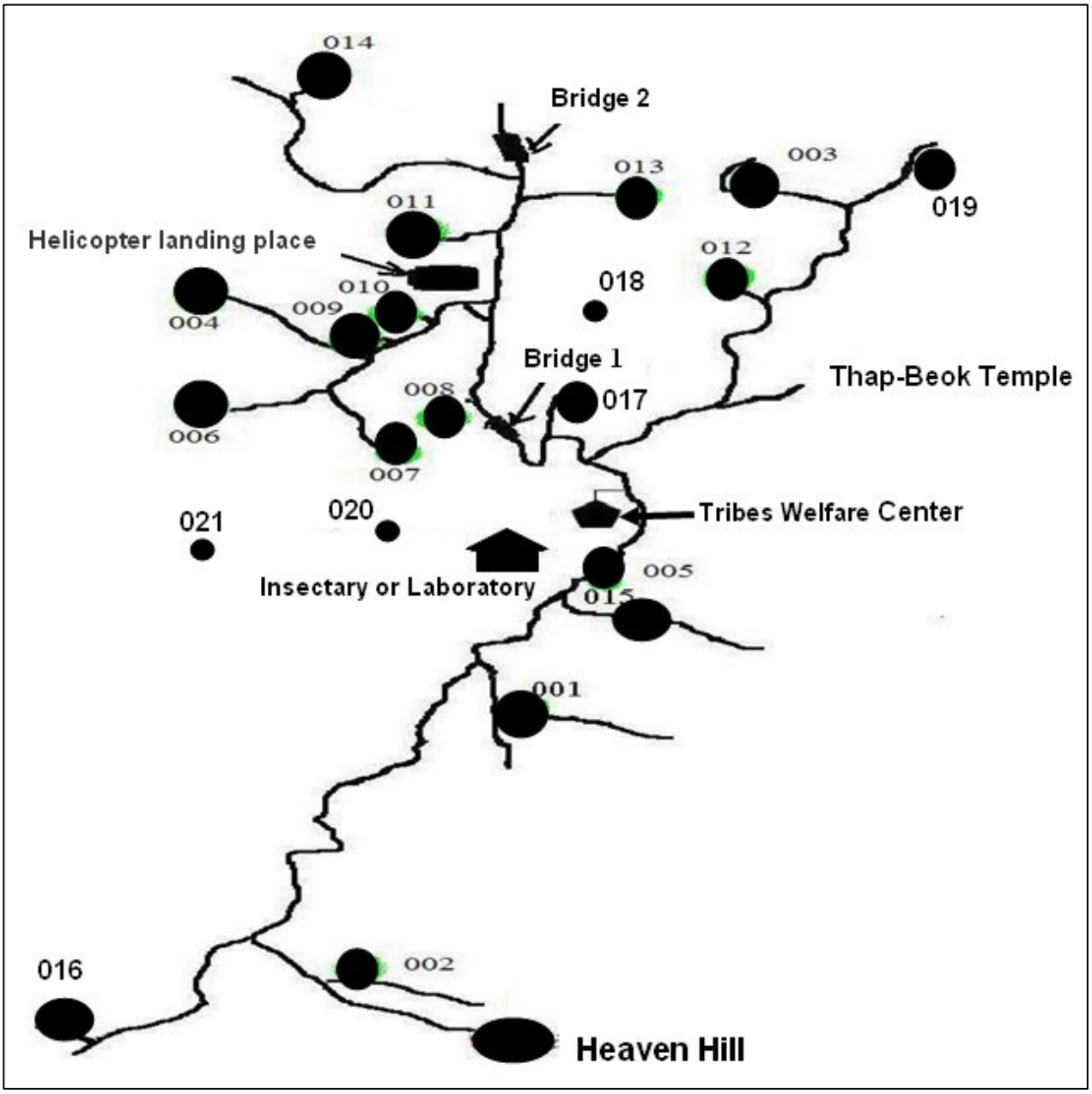

Figure 1. Map showing where *Diadegma semiclausum* was released and successfully established in 21 sites (001-021) in Thap Boek (Phetchabun province) as of year 2010.

unacceptable pesticide residues, and thus are able to gain access to more lucrative domestic and international markets. That *D. semiclausum* after its introduction and establishment in Thailand is able to offer such immense benefits to farmers who devotedly practice IPM to conserve the parasitoid is no surprise as this has occurred over and over again in several highlands of tropical countries in the region, for example, those in Indonesia (Sastrosiswojo and Sastrodihardjo 1986), the Cameron Highlands in Malaysia (Ooi and Lim 1989; Ooi 1992; Syed et al. 1997), Cordillera Highlands in the Philippines (Poelking 1992; IIBC 1996; Ventura 1997), and Da Lat in South Vietnam (Ooi et al. 2001; Nga and Kumar 2008).

In more recent years, parasitoids were also released into the fields of non-FFS farmers who commonly apply chemical insecticides to control DBM in their fields. It is hoped that the released parasitoids, plus those which have spread there naturally from FFS fields, would take their natural course to become established over time in these non-FFS fields. Such a process, however, would require substantially longer time because of the adverse impact of the pesticides applied by these farmers. And this is observed to have started to happen as *D. semiclausum* can now be seen in a number of non-FFS fields, albeit the level is still very low and insufficient to exert significant control of DBM there. With continuing expansion of IPM-FFS training to more and more farmers there, it is envisaged that *D. semiclausum* will before long become the mainstay for biological control of DBM in the Phetchabun highlands, thereby resulting in improved crucifer farming that follows good agricultural practices to produce healthy and safe vegetables.

CONCLUSION

That *D. semiclausum* can readily and quickly establish area-wide in intensively-sprayed crucifer production areas in the Phetchabun highlands in north-eastern Thailand clearly illustrates the importance of releasing parasitoids in conjunction with IPM-FFS training. This is especially so in situations where the farmers normally rely exclusively on chemical insecticides to control their crop insect pests, whereby any parasitoids introduced would likely succumb to the chemicals that are applied in the field. Hence, coupling the parasitoid releases with IPM-FFS training is crucial to educate farmers on the role of the parasitoids, including how to avoid or minimize the adverse impacts of pesticides to provide the best chance for the parasitoids to survive, and for their speedy establishment in the field.

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Control management of diamondback moth, *Plutella xylostella* (Linnaeus) in Chinese kale

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ABSTRACT
Management of diamondback moth, *Plutella xylostella* was tested both in laboratory, Department of Agriculture and in farmer's field at Amphor Thamuang, Kanchana Buri province during 2008–10. Toxicity of some selected insecticides was tested against the larval parasitoid, *Cotesia plutellae* in the laboratory. It was found that flubendiamide (Takumi 20% WDG) at the rate of 6 g/20 l of water and Bt (Xentari WDG) at the rate of 4 g/l of water were harmless to adults of *C. plutellae* with 6.7 and10% mortality, respectively; however, tolfenpyrad (Hachi Hachi 16% EC) at the rate of 1.5 ml/l of water was slightly harmful recording 60% mortality. Field trials were carried out to test the best alternate sprays in Chinese kale growing area before making decisions whether to spray or not to spray. At the economic threshold level of 1.25 larvae per 10 plants, the alternate spray of two insecticides and Bt were sprayed. At less than 1.25 larvae per 10 plants, no spraying was given; at more than 1.25 larvae per 10 plants, spraying with Bt and alternate spraying with flubendiamide and tolfenpyrad were performed. The average number of DBM in experimental field was 2.19 larvae per 10 plants with five sprayings of chemical insecticides in alternate with two applications of Bt. In the farmers' fields, which received eight applications of DBM resistant insecticides, the average number of DBM were 7.38 and 6.48 larvae per 10 plants and the yield and grade were lower in these fields. To extend the methodology, farmers' meeting was held in the vegetable growing district. They accepted how to do alternate sprays and scouting before spraying to decrease and slow down DBM insecticide resistance development.

Keywords
Diamondback moth, *Cotesia plutellae*, insecticide resistance, alternate sprays, economic threshold level

INTRODUCTION
Chinese kale (*Brassica alboglabra* Bailey) is an economically important Crucifer crop with a growing area of 14,800 ha, yielding 142,000 t in Thailand (OAE 2009).The most serious pest of Chinese kale is diamondback moth, *Plutella xylostella* Linnaeus. The larvae cause severe damages to the leaves, and because of the tropical climate, it has shorter lifecycle and thus producing several generations in a year. For instance, DBM completes its lifecycle in 17-18 days during summer (April-May) and 29 days in winter (November – December). Hence, it could produce 17–25 generations in a year (Piyarat et al. 1988).

Vegetable is being grown throughout the country in Thailand. Most vegetable growers are small holders. They grow the same crucifers that provide more profit, continuously. Since DBM is a persistent problem, the farmers apply the chemical pesticides continuously. There were several studies documenting the efficacies of various pesticides. For example, flubendiamide showed good result followed by *B.t. aizawai* and *B.t. kurstaki* against Tamuang strain of DBM (Suprada et al. 2009). In recent years, diamide group of insecticides such as flubendiamide (Takumi 20% WDG) and chlorantraniliprole (Prevathon 5% SC) showed good results, In addition, tolfenpyrad (Hachi Hachi 16% EC), *B.t. aizawai* (Xentari) and spinosad (Success 12% SC) also provided effective control of DBM (Jeeranut et al. 2009). However, intensive use of pesticides led to the development of pesticide resistance. The new effective insecticides have become ineffective within 2-3 years of their introduction. Insecticide resistance monitoring was done in Nontha Buri province during 1998-99. Insecticides like fipronil had been put aside by the growers because Bangbuathong or Sainoi strain of DBM became highly resistant (Parnpen et al. 2001).

Insecticide Resistance Action Committee (IRAC) has suggested to use economic threshold level and alternate sprays with different group or modes of action insecticides to slow down the development of resistance. Hence, insecticide resistance management strategy should be developed in a way that would be acceptable to

the farmers. We report the development of alternate spray strategy in this paper. In addition, effects of harmless or slightly harmful insecticdes were also evaluated against the parasitoid, *Cotesia plutellae* Kurdjumov.

MATERIALS AND METHODS

Evaluation of insecticide toxicity against Cotesia plutellae

Mass rearing of DBM

The DBM larvae were collected from cabbage fields. Mass rearing of DBM was done in the laboratory condition at 25±1°C, 70% RH and photoperiod 16:8 h. (light:dark). The Chinese kale seedlings were put into the insect cages as egg laying substrate for DBM adults. The cages contained DBM adults collected from the fields. The cotton pad dipped in 10% honey solution was supplied as food for adults. At 7 days after egg hatching, the larvae attained 2nd instar on the Chinese kale seedlings. The larvae were divided into two groups. The first group was used to rear *C. plutellae*. The other group was used to maintain stock culture of DBM for the next round of rearing.

Mass rearing of C. plutellae

The cocoons of *C. plutellae* were collected from cabbage fields. The mass rearing of *C. plutellae* was conducted in the laboratory condition at 25±1°C and 70% RH. The emerged adults from the cocoons were put into the cages containing 2nd instar larvae of DBM on the Chinese kale plant. After 15 d, the cocoons of *C. plutellae* were collected and used in the experiments. Some part of *C. plutellae* adults were used as stock culture for the next round of rearing. The experimental design was Completely Randomized Design with three replications using 11 insecticides and control. The insecticides were methoxyfenozide (Prodigy 24% SC), spinosad (Success 12% SC), indoxacarb (Ammate 15% SC), emamectin benzoate (Proclaim 1.92% EC), chlorfenapyr (Rampage 10% SC), fipronil (Ascend 5% SC), lufenuron (Match 5% EC), tolfenpyrad (Hachi Hachi 16% EC), flubendiamide (Takumi 20% WDG), neem product 0.1 % w/v and B.t. (Xentari WDG).)The one-day old parasitoids were tested with determined concentration of each insecticide using dry film method. Acetone was used to dilute each insecticide. The 0.2 ml of each diluted insecticide was dropped inside the test tubes (1.5 cm in diameter and 10 cm long). The test tubes were gently rolled to produce uniformly insecticide-coated wall, and left for 2 h until acetone was completely evaporated. The control used only acetone to coat the inside wall of test tubes. Ten adults were used for each treatment. One adult parasitoid was released into each insecticide-coated test tube. The cotton soaked with 15% honey solution was supplied at the nylon cap of test tube as the food source for the parasitoids. The mortality of parasitoids was checked at 12, 24, 48 and 72 h. If control showed mortality, the mortality percentage was adjusted with the Abbott's formula (Abbott 1925), and the corrected mortality percentage was used for statistical analysis.

The toxicity of insecticides against natural enemy was assessed using the following grouping (Hassan et al. 1994):

Insecticide causing	Category	Rating scale
less than 30% mortality	Harmless	1
30-79 % mortality	Slightly harmful	2
80-99 % mortality	Moderately harmful	3
more than 99% mortality	Harmful	4

Field trial on the management of DBM

Alternate sprays were given in the field trial with Chinese kale. The number of DBM larvae from 100 sampling plants was counted before spraying. Sampling was done at every 4-5 days interval. Economic Threshold (ET) was 1.25 larvae/10 plants; if the mean number of DBM larvae is >ET to 1.5 per 10 plants, the field was sprayed with Bt; >1.5 larvae/10 plants, it was sprayed with insecticides in rotation (alternate). Samplings of DBM were also done in farmers' fields, which served as control. Both yield and grading were done. The following grading was used:

Grade A : no damage
Grade B :1-20% damage
Grade C : 21-50% damage
Grade D : >50% damage
Grade E : 100% damage

RESULTS AND DISCUSSION

Evaluation of insecticide toxicity against *Cotesia plutellae*

The harmless insecticides (Rating scale 1) to *C. plutellae* were flubendiamide, neem extract, emamectin benzoate, lufenuron, Bt, methoxyfenozide and spinosad recording 6.70, 2.00, 3.30, 6.70, 10.00, 3.30 and 16.70% mortality, respectively, which were not significantly different from the control (3.30%) at 24 h after treatment. For 48 h after treatment, these insecticides caused 10.00, 6.00, 6.70, 10.00, 13.30 and 13.30% mortality. However, spinosad at 48 h caused 33% mortality, which was significantly higher than the control. Spinosad was categorized in rating scale 2 (Tables 1 and 2) and classified as slightly harmful according to Hassan et al. (1994).

Field trial on the management of DBM

From the laboratory studies conducted earlier, the most effective insecticide for controlling DBM was flubendiamide, followed by *B.t. aizawai* and *B.t. kurstaki* (Sukonthapirom et al. 2009). The most effective insecticides from earlier field trials were flubendiamide,

followed by chlorantraniliprole, tolfenpyrad and B.t. (Aekamnuay et al. 2009). Since those pesticides were categorized under different group or modes of action, they were used in alternate spray (rotation) in the field trial with Chinese kale at 30 days old. Since the first counting showed a mean larval population of 0.2 per 10 plants, no spraying was given. However, second and third countings recorded a mean population of 5 and 1.1 larvae/10 plants; hence, flubendiamide and B.t. were applied, respectively. In the subsequent countings, the mean larval populations were 3.3, 1.5 and 2.8 larvae/10 plants; hence, flubendiamide was sprayed in alternate with tolfenpyrad. The last counting recorded a larval population of 0.7 per 10 plants and B.t. was applied (Table 3 and Fig.1). The mean number of DBM larvae in the demonstration field was 0.2 larvae/10 plants compared to two farmers' fields recording 7.38 and 6.48 larvae/10 plants. The yield from demonstration field was 7.71 (Grade A), 11.38 (Grade B), 5.60 (Grade C) and 0.19 (Grade D) t/ha, whereas the first farmer got only 0.72 (Grade C) t/ha and the second farmer got 0.51 (Grade A), 2.07 (Grade B), 6.36 (Grade C) and 8.89 (Grade D) t/ha (Table 4 and Fig.2)

CONCLUSION

The harmless insecticides to *C. plutellae* and effective for controlling DBM were flubendiamide and B.t. The slightly harmful insecticide against the parasitoid was tolfenpyrad. In the demonstration field, based on Economic Threshold (1.25 larvae/10 plants), flubendiamide alternated with tolfenpyrad was sprayed for five times, and B.t. was sprayed for two times depending on the number of DBM larvae. The yield of demonstration field was 24.880 t/ha, with Grade A yield of 7.71 t/ha, compared to farmers' fields that were sprayed for eight times with chemical insecticides recording a yield of 17.83 t/ha with Grade A yield of only 0.51 t/ha.

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Table 1. Mortality of *Cotesia plutellae* caused by various insecticides and Bt. in laboratory

Treatments	Rate/20 l of water	Mortality (%)after	
		24 h	48 h
Methoxyfenozide	40 ml	3.30 a	13.30 ab
B. t. aizawai	80 g.	10.00 a	13.30 ab
Spinosad	40 ml	16.70 a	33.30 b
Indoxacarb	15 ml.	96.70 c	100.00 c
Emamectin benzoate	20 ml	3.30 a	6.70 ab
Chlorfenapyr	40 ml	90.00 bc	100.00 c
Fipronil	60 ml	100.00 c	100.00 c
Lufenuron	20 ml	6.70 a	10.00 ab
Tolfenpyrad	30 ml	60.00 b	83.30 c
Flubendiamide	6 g	6.70 a	10.00 ab
Control (acetone)	5 ml	3.30 a	3.30 a

The values followed by same letter(s) in a column are not significantly different

Table 2. Mortality of *Cotesia plutellae* caused by neem extract and B. t. in laboratory

Treatments	Rate/20 l of water	Mortality (%) after	
		24 h	48 h
Neem extract	100 ml	2.00a	6.00a
B. t. aizawai	80 g	2.00 a	10.00 a
Control (acetone)	5 ml	0.00a	2.00a

The values followed by same letter(s) in a column are not significantly different

Table 3. Number of DBM larvae in the field trial before starting the alternate spraying at Tamuang, Kanchana Buri province, Thailand

Spray time	Mean no. of DBM larvae per 10 plants	Rate/20lof water
-	0.20	No spray
1	5.00	Flubendiamide (Ta) 6g
2	1.10	Bt 80g
-	0.70	No spray
3	4.80	Tolfenpyrad (Ha) 30 ml
4	3.30	Flubendiamide (Ta) 6g
5	1.50	Tolfenpyrad (Ha) 30 ml
6	2.80	Flubendiamide (Ta) 6g
7	0.30	Bt 80g

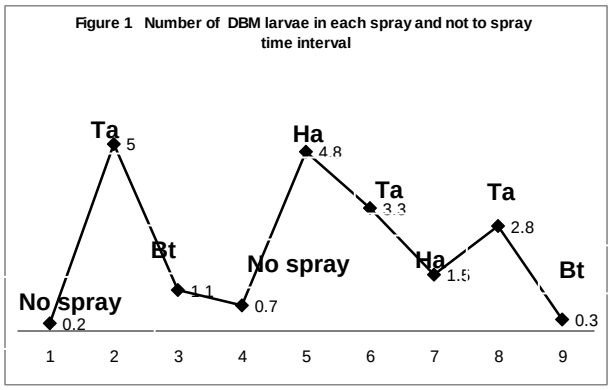

Figure 1. Number of DBM larvae in each spray and no spray time interval

Table 4. Number of DBM larvae and yield in field trial and in farmers' fields at Tamuang, Kanchana Buri province, Thailand

List	demonstrated field	Farmer' field 1st	Farmer' field 2nd
Average No. of DBM per10 plants	2.19	7.38	6.48
Yield (A) t/ha	7.71	-	0.51
Yield (B) t/ha	11.38	-	2.07
Yield (C) t/ha	5.60	0.72	6.36
Yield (D) t/ha	0.19	-	8.89
Yield (E) t/ha	-	-	2
chemical spray	5 times	8 times	8 times
B. t. spray	2 times	-	-

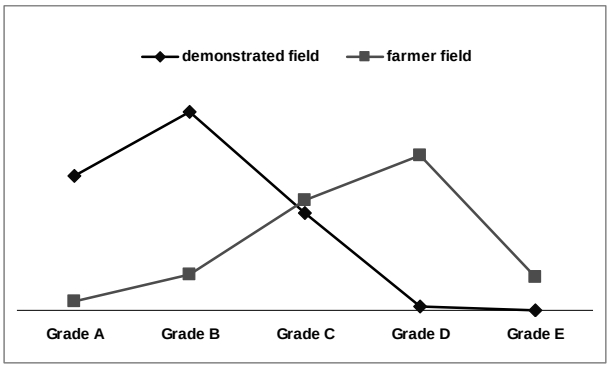

Figure 2. Yield and quality of Chinese Kale in demonstration field and farmers' fields at Tamuang, Kanchana Buri province

Status, damage potential and management of diamondback moth, *Plutella xylostella* (L.) in Tamil Nadu, India

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ABSTRACT
The diamond back moth (DBM), *Plutella xylostella* (L.) is an economically important pest of crucifers in Tamil Nadu, India. The biology of *P. xylostella* varies significantly with cruciferous crops and most preferred host is cauliflower. Population of DBM is influenced by abiotic factors, including temperature, rainfall and humidity. The population becomes abundant during September to October and March to April. Mustard as a trap crop is planted alternatively with every 25 rows of cabbage and cauliflower. The second and third instar larvae of DBM are naturally parasitized by *Cotesia plutellae* (Kurdy) and *Diadegma semiclausam* (Hellen) in plain and highland areas, respectively. Microbials *viz., Bacillus thuringiensis* (*B.t*), *Beauveria bassiana*, *Paecilomyces fumosoroseus, Metarhizium anisopliae* and baculoviruses (Granulo viruses and NPV) are alternatives to chemical pesticides to control this pest. Use of novel insecticides, growth regulators and botanicals *viz*., indaxocarb (29 g a.i./ha), abamectin (15 g a.i./ha), emamectin @ 10 g a.i./ha, thiodicarb (1.25 kg / ha), cartaphydrochloride (1 kg/ha), spinosad 2.5 SC @ 15-25 g a.i./ha, spinosad (15 g a.i./ha), emamectin benzoate (5% S G)150-200 g/ha, novaluron (0.0075% or 0.75 ml/l), lufenuron (0.012 %), neem oil 2 % and NSKE 5 % are effective in checking the DBM larval population. DBM has developed resistance to all chemical insecticides and Bt in India. Monitoring the insect resistance development to new molecules of chemical pesticides provides a way to delay resistance development.

Keywords Pest status, damage potential, *Plutella xylostella*, management, Tamil Nadu.

INTRODUCTION
Cabbage, *Brassica oleracea* var. *capitata* L. and cauliflower, *B. oleracea* var. *botrytis* L. are important crucifers in India with an area of 2,32,800 and 2,78,800 hectares, respectively. The diamondback moth (DBM), *Plutella xylostella* (Linnaeus) (Plutellidae: Lepidoptera), is the major destructive pest on cruciferous crops such as cauliflower, cabbage, and mustard, and causes significant economic losses (92% farmers) up to 50% with an estimate of US$ 168 million per year.

Most often growers resort to prophylactic and scheduled applications of chemical insecticides. Consequently, problems like resurgence, resistance, residues, replacement, destruction of nontarget organisms and environmental pollution have been on the rise. DBM has become very difficult to manage because of the development of high levels of resistance to several groups of organophosphorus, carbamate, and pyrethroid insecticides. Sole reliance on insecticides has facilitated rapid build-up of resistance in the multivoltine DBM, which undergoes 20 generations a year in the tropics (Talekar and Shelton 1993). To overcome resistance in DBM to insecticides, farmers often increase the doses of insecticides when insecticides alone account for between 30 and 50% of the total cost of production. Health problems with farmers were also common in states where these crops are grown (Weinberger and Srinivasan 2009).

Distribution
DBM has its origin either in the Mediterranean region or in South Africa in 1890. In India, DBM was reported in 1914 on cruciferous vegetables and is now the most devastating pest of cole crops in the states of Punjab, Haryana, Himachal Pradesh, Delhi, Uttar Pradesh, Bihar, Tamil Nadu, Maharashtra and Karnataka. Sachan and Gangwar (1980) studied the vertical distribution of this pest from the higher altitude ranges of Mehalayas (Fig. 1).

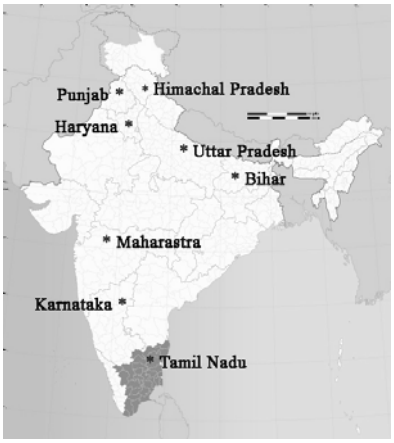

Figure 1. Geographical distribution of diamondback moth in India.

Pest status and damage potential

The diamondback moth, *P. xylostella*, has consistently remained the most destructive insect pest of crucifer vegetables worldwide. Major outbreaks of *P. xylostella* are more likely in the fields that are sprayed frequently and heavily with insecticides. The absence of effective natural enemies and fast development of insecticide resistance are believed to be the major causes of increasing pest status of *P. xylostella* in most parts of the country. This insect is resistant to many classes of insecticides and causes 50 to 80% loss in marketable yield. An outbreak of DBM on cauliflower was reported in Aligarh during September to first fortnight of October 2006. The infestation increased gradually from first fortnight of August and led to total loss of the crop. Climatic changes may lead to increase in severity of this pest in many regions of the country (Dhaliwal et al. 2010).

Host plants

DBM in India infests important cultivable crucifers *viz.,* cabbage, cauliflower, broccoli, radish, turnip, Brussels sprout, Chinese cabbage, knol-khol, rabi mustard, rape seed, collard, patchouli, saishin, watercress and kale. Studies on the food plant preference of DBM revealed that the larvae have a marked preference for cauliflower and cabbage among the 39 species of crucifers. However, larval and pupal periods varied among cauliflower, turnip, cabbage, radish and mustard, while the survival rate was the greatest on cabbage. This is due to the fact that both plants possess fleshy and succulent leaves compared to rest of the crucifers tested, and probably provides olfactory and gustatory stimuli for successful host selection and development. Non-cruciferous crops acting as alternate hosts include garlic, onion, sugar beet, okra, and amaranthus (Kalyanasundaram 1995).

Biology

The biology of this pest has been studied in the laboratory and under natural conditions in relation to ecological factors (Kalyanasundaram 1995). Eggs are laid in groups usually on the adaxial surface of leaves near veins and occasionally on both surfaces. The eggs are minute, whitish yellow and 0.5 mm long and each female could lay 164 eggs under field conditions. Egg period varies between two and six days. Neonates are pale white with dull brown head while fully grown caterpillars are light green in colour measuring 10 mm in length. Short hairs become visible on green mature larvae which wriggle violently on slightest touch. The larvae feed for a variable period of time extending between 14 and 21 days before pupation. First instar larvae mine into leaves until the first moult, after which they feed externally and have four larval instars. Male larvae are distinguished from the female by the presence of white conspicuous gonads on the dorsum of the fifth abdominal segment of last instar. Pupation occurs near the midrib on the abaxial surface of the leaf in a gauze like silken cocoon loosely spun by the caterpillar. Pupa is 6 mm long and light brown in color. Pupal period lasts for four days in hot and rainy season, but five days in cold season.

Moths are small greyish with a wing expanse of 14 mm. The wing of male moths is folded outward and upward towards their tips forming a row of three diamond-shaped yellow spots along the middle of the back. Adult longevity ranges from 6-13 days, females live shorter than males. The ratio of males to females is often 1:2. Adults begin to mate at dusk on the same day of emergence; mating lasts one to two hours and females mate only once. Females lay eggs after mating and oviposition continues for 10 days with a peak on the first day of emergence. Two to four generations occur in colder parts of India and 13-14 generations occur in South India.

Comparative biology

The biology significantly varies on cauliflower, cabbage, mustard, radish, knol-khol, turnip, beetroot, amaranthus and weed host. There is no variation in the egg period. Larvae develop much faster (8.5 days) and pupate on cauliflower. Pupal period is shorter and adult emergence is more on cauliflower. Fecundity is higher on mustard (366 eggs/female), followed by cauliflower, cabbage, radish and knol-khol (Table 1). The larvae consumed and gained more weight on cauliflower than on other host plants. The consumption index, growth rate, efficiency of conversion of ingested food, efficiency of conversion of digested food and approximate digestibility were more on cauliflower than on other host plants (Kalyanasundaram 1995).

Seasonal incidence

Seasonal incidence of the pest on cabbage varied among locations in Tamil Nadu (Udhagamandalam, Coimbatore, Hosur, Ottanchatram, Theni and Tenkasi). The larval population was highest in Ottanchatram, lowest in Tenkasi. Population of DBM is generally influenced by abiotic factors like, temperature, rainfall and humidity. The population became abundant during September to October and March to April. Heavy rain can destroy the moth population. High build-up of larval populations has been reported during February-March (late winter) and April-August (summer and mid rainy season) (Abraham and Padmanabhan 1968). However, Jayarathnam (1977) found significantly high build-ups of larval populations during the rainy season (July- September) compared with other seasons.

Kalyanasundaram (1995) reported that the extent of parasitisation (%) was more by *C. plutellae* than *D. semiclausam* on DBM among locations (Table 2). Jayarathnam (1977) studied the population dynamics of the pest by constructing life tables for 10 generations (five in rainy season and five in cold season). Major mortality factors were parasitization by *A. plutellae* in the larval period, predatory ants, birds and spiders in the 1st and 2nd larval instars, and rainfall in the 1st and 2nd larval

instars (Table 3). The major mortality factor at the pupal stage was parasitization by *T. sokolowskii*. Parasitization by *T. sokolowskii* was identified as the key mortality factor in all 10 generations. Infestation by the cabbage webworm, *Hellula undalis* Zell, resulted in considerable reduction of oviposition sites for DBM.

Table 1. Effect of host plants on biostages of *P. xylostella*

Host plant	Egg period (days)	Larval period (days)	Pupation (%) P	Larval growth index P	Pupal weight (mg)	Pupal period (days)	Adult emergence (%)	Fecundity (No. eggs/female)
Cauliflower	3.0	8.5	94.2	11.08	260	5.6	85.4	346
Cabbage	3.2	9.0	92.4	10.27	258	5.7	82.7	342
Mustard	3.0	9.2	88.6	9.63	256	6.0	81.6	366
Radish	3.0	9.7	85.4	8.80	250	6.0	78.1	333
Knol-khol	3.4	10.8	84.7	7.84	243	6.2	75.8	320
Turnip	3.0	11.4	80.6	7.07	238	6.4	74.6	295
Beet root	3.5	12.8	78.4	6.13	216	6.6	73.2	273
Amaranthus	3.5	14.6	76.5	5.24	200	8.0	70.4	240
Cleome gynandra	3.5	15.4	68.0	4.44	181	8.2	65.6	186

Table 2. Parasitism by larval parasitoids of DBM in the field

District	Locality	Mean larvae /Plant	Parasitism (%)							
			C. plutellae				*D. semiclausum*			
			40 DAP	55 DAP	70 DAP	Mean	40 DAP	55 DAP	70 DAP	Mean
Coimbatore	Alanthurai	8.64	07.20	11.60	16.40	11.70	00.00	00.00	00.00	00.00
Nilgiris	Udhagamandalam	13.87	03.00	07.10	06.20	05.40	02.00	05.10	07.20	04.88
Dharmapurai	Hosur	10.43	05.10	17.90	28.30	17.10	00.00	02.60	07.20	02.10
Dindigul	Ottanchatram	16.07	17.10	38.50	31.00	28.92	00.00	00.00	03.10	00.00
Tirunelveli	Tenkasi	6.43	01.70	06.60	10.40	06.21	00.00	00.00	00.00	00.00

DAP: Days After Planting

Table 3. Mortality factors of DBM

Stage	Rainy season		Winter season	
	Mortality factors	Mortality (%)	Mortality factors	Mortality (%)
Egg	Infertility	6.6	Infertility	14.3
I instar	Rainfall (39 mm) Predators: ants and spiders	11.0 59.3	Predators: ants, birds, and spiders, heavy dew	60.0
II instar	*A. plutellae* Predators: ants and spiders	52.0 13.9	*A. plutellae* Predators: ants, birds, and spiders	20.0 59.2
III instar	*T. sokolowski*	4.0	*T. sokolowski*	8.0
Pupa	*T. sokolowski*	32.0	*T. sokolowski*	72.0
Adult	Sex: 45% female	9.2	Sex: 41% female	18.0

Life table

Life table of DBM reveals that many larvae died during immature stage. The key mortality factors during summer season were mainly natural enemies, precipitation and high temperature. The life table of *P. xylostella* on cauliflower indicated that 23% mortality occurred in the egg stage. The two-day pre-oviposition period was between 20 and 21 days of pivotal age. Adult moths laid eggs between 22 and 31 days. Female moths started dying one day after the adults had emerged (1x 0.70). Mortality increased thereafter. Adults attained greatest daily mean fecundity (mx of 20.07) on 27th day of pivotal age. Reproduction ceased ten days after oviposition. The net reproductive rate (Ro) representing the total female births was ($\sum$ 1x. max) 92.86 (Jayarathnam 1977 and Kalyanasundaram 1995).

CULTURAL CONTROL

Cultural practices have had significant impact on DBM management. Removal of alternative hosts, volunteer plants and crop residues could disrupt the development of DBM. Fallowing and land drying would clean up sources of DBM as well as improve general soil conditions. Regulated irrigation with sprinklers has reduced DBM infestations. Adjusting the time of planting and adopting crop rotation are other eco-friendly approaches in managing DBM. As DBM infestations are generally lower during the rainy season, Lim (1982) suggested that cultivation of crucifers in the drier parts of the year should be avoided to minimize the incidence. In large commercial cultivation, crop rotation of crucifers with cucurbits, beans, peas, tomato, and melons could suppress DBM population substantially. Many wild flowering plants and non-cruciferous crops like legumes support natural enemies by providing nectar and pollen.

Trap cropping

Growing of two rows of mustard after every 25 rows of cabbage as a trap crop reduced 80-90% of DBM population and other pests. Mustard should be sprayed with Dichlorovos 0.1% as soon as it germinates. (One row of mustard is sown 15 days before cabbage planting and a second row 25 days after planting of cabbage). First and last row of plots also should be mustard. DBM colonized on mustard, sparing the main cabbage crop (Srinivasan and Krishnamoorthy 1992).

Intercropping

Tomatoes when intercropped with cabbage at 1:4 ratio significantly reduced DBM. Sivapragasam et al. (1982) stated that intercropping of tomatoes with cabbage at 1:4 ratio significantly reduced DBM infestation by 36%. On the other hand, more number of eggs and adults of *P. xylostella* were counted on cabbage when it was raised as a pure crop. Similarly, intercropping in cauliflower (4:1), especially with tomato and onion, significantly minimized the DBM larval population. The mean number of larvae that occurred on tomato intercropped with cauliflower (8.23/plant) was significantly fewer than on the pure crop (16.13/plant). On the other hand, the mean parasitism by *C. plutellae* was maximum on tomato intercropped cauliflower (29.61%), nearly 50% higher than that on the pure crop (Kalyanasundaram 1995) (Table 4).

Table 4. Impact of intercrops in cauliflower on DBM infestation and parasitisation by *C. plutellae*

Crop combination	Number of DBM larvae/plant on DAP			Parasitisation (%) on DAP		
	40	55	70	40	55	70
Cauliflower + Onion (4:1)	7.31	12.64	9.75	14.20	21.30	30.61
Cauliflower + Tomato (4:1)	6.84	10.24	8.23	22.30	28.10	38.45
Cauliflower + Mustard (4:1)	16.48	17.86	13.41	11.60	10.87	20.46
Cauliflower + Cluster beans (4:1)	11.46	14.13	10.00	11.60	12.80	21.00
Cauliflower alone	16.13	21.36	14.40	10.60	14.41	23.83

DAP: Days after planting

BIOLOGICAL CONTROL

Predators, parasitoids and pathogens may play an important role in suppressing DBM populations.

Predators

Among the predators spiders, syrphids, wasps, coccinellid beetles, penatomid bugs, phytoseiulus mites, chrysopids, Ophionea beetle and bird predators have been observed to build up only in the later phase of the crop, causing as much as 68-70% larval mortality. Although predators have been suggested as mortality factors, they have not been commercially exploited against DBM (Lim 1982).

Parasitoids

P. xylostella eggs, larvae, pupae and adults are devoured by numerous natural enemies. As far as parasitoids are

concerned, studies are much limited. Larval parasitoids are the most predominant and most effective. The most efficacious larval parasitoids belong to two major genera, *Diadegma semiclausum* and *Cotesia* (=*Apanteles*). *C. plutellae* was found causing 16 to 70% larval parasitism in India. *D. semiclausum* was also observed successfully parasitizing in the highlands of Tamil Nadu. Barring these reports, little information is available on the commercial exploitation of DBM parasitoids in India (Table 5).

Table 5. Natural enemies of DBM in India

Sl. No.	Species	Stage	Reference
I.	**PARASITOIDS**		
1.	*Trichogramma chilonis* Ishii (Trichogrammatidae : Hymenoptera)	Egg	Anuradha (1997)
2.	*Trichogramma armigera* Nagaraj (Trichogrammatidae : Hymenoptera)	Egg	Manjunath (1972)
3.	*Trichogrammatoidea bactrae* Nagaraj (Trichogrammatidae: Hymenoptera)	Egg	Singh and Jalali (1993)
4.	*Cotesia (Apanteles) plutellae* (Braconidae : Hymenoptera)	Larvae	Nagarakatti and Jayanth (1982)
5.	*Diadegma fenestrate* Holmgren *Diadegma collaris* Graven horst	Pupa	Chauhan et al. (1997) and Devi and Raj (1995)
6.	*Diadegma semiclausum* Horstmann	Pupa	Chandramohan (1994)
7.	*Tetrastichus sokolowskii* Kundj (Eulopidae : Hymenoptera)	Larval	Nagarakatti and Jayanth (1982)
8.	*Brachymeria exacarinata* Gahan (Chalcididae : Hymenoptera)	Pupa	Cherian and Basheer (1938)
II.	**PREDATORS**		
1.	*Chrysoperla cornea* Stephens (Chrysopidae : Neuroptera)	Egg & Larva	Anuradha (1997)
2.	*Coranus sp*. (Reduvidae : Hemiptera)	Larva	Anuradha (1997)
	Ant		
1.	*Tapinoma melanocephalum* (Formicidae : Hymenoptera)	Larva	Jayarathnam (1977)
2.	*Componatus sericus* (Formicidae : Hymenoptera)	Larva	Jayarathnam (1977)
3.	*Pheidole sp*. (Formicidae : Hymenoptera)	Larva	Jayarathnam (1977)
	Birds		
1.	Yellow wag tail (*Motacilla flava*)	Larva	Jayarathnam (1977)
2.	Cattle egret (*Bulbueus ibis*)	Larva	Jayarathnam (1977)
III.	**PATHOGENS**		
1.	*Bacillus thuringiensis* var. Kurstaki	Larva	Narayanan et al. (1970)
2.	Nuclear polyhedrosis virus (NPV)	Larva	Anuradha (1997)
3.	Granulosis virus (GV)	Larva	Rabindra et al. (1996)
4.	*Paecilomyces farinosus* (Fungus)	Larva	Anuradha (1997) and Gopalakrishnan (1998)
5.	*Beauveria bassiana* (Fungus)	Larva	Voon et al. (1999)
6.	*Zoophthora radicans* (Fungus)	Larva	Gopalakrishnan (1998)
7.	*Varirіomorpha* sp. (Protazoa)\	Larva	Anuradha (1997)
8.	Nematode	Larva	Anuradha (1997)

Biopesticides

Microbial pesticides (Bt, fungi and viruses) offer high potential for delaying the development of resistance. These are more effective when used in combination with chemical insecticides. Entomopathogenic fungi and bacteria paralyze or kill their host by adversely affecting growth and development of host insects.

*Bacillus thuringiensis (*Bt*)*

Currently, products based on Bt are the most successful microbial pesticides accounting for more than 90% of biopesticide sale. Many commercial formulations containing high levels of α-endotoxins have proven to be as effective as chemical insecticides. In the laboratory, bioassays were conducted to determine the efficacy of commercial products. In many instances Bt was either superior to chemical insecticides or equally effective. Narayanan et al. (1970) studied the effectiveness of Bt (Thuricide) in the form of dust, wettable powder or emulsion for the control of DBM under laboratory, field and glasshouse conditions. Mohan and Gujar (2000) reported that among the Bt formulations tested, Biobit© and Delfin© were more effective (LC_{50} 1.49 mg a.i./l and $0.6X10^6$ SU/l respectively) than Dipel©, HIL© and Halt©. Baseline susceptibility of *P. xylostella* populations collected from 13 geographic locations to Biobit® revealed that the population obtained from Pune was the most susceptible (LC_{50} 1.01 mg a.i./l) and Iruttupallam field population was least susceptible (LC_{50} 10.97 mg a.i./l) than Ottanchatram (6.78 mg a.i./l). Compared with the most susceptible field population (Pune), the Iruttupallam population was 10.86-fold more resistant. Laboratory selection of diamondback moth with Biobit® increased insect resistance. Complete bioassays were carried out to confirm development of resistance for populations belonging to F_1, F_5 and F_9 generations. The LC_{50} increased from 2.76 mg a.i./l to 4.62 mg a.i./l and 5.28 mg a.i./l in F_1, F_5 and F_9 generations respectively. Toxicity of Cry proteins *viz*., Cry1Aa, Cry1Ab, Cry1Ac and Cry1C were tested on *P. xylostella.* Among the Cry1A toxins, Cry1Aa showed less toxicity by two orders of magnitude as compared to Cry1Ab (Mohan and Gujar 2000).

Baculoviruses of P. xylostella

Granulovirus (GV) was the first baculovirus reported from the larvae of *P. xylostella* (PXgv). The pathology of the disease, host interactions, field efficacy and potential use of GV as a biocontrol agent against *P. xylostella* have been extensively reviewed. Nuclearpolyhedrovirus of *P. xylostella* (PxNPV) was first observed in cauliflower fields of India (Rabindra et al. 1996). NPV from *P. xylostella* was cross-infective to several lepidopteran pests. Kadir et al. (1999) reported the susceptibility of *P. xylostella* to NPV of *Anagrapha falcifera* (Kirby) (AfMNP) and *Autographa californica* (Speyer) (AcMNPV). AcMNPV was found to be more virulent than AfMNPV for *P. xylostella*. Neonates of *P. xylostella* were found to be highly susceptible to GmNPV with low LT_{50} when compared to that of AcMNPV and PxGv. Cross-infectivity studies at Tamil Nadu Agricultural University, Coimbatore with GmNPV revealed the susceptibility of 16 insect species (Parthasarathy 2002). Second, third and fourth instar larvae of *P. xylostella* were susceptible to two isolates of GmNPV *viz*., Coimbatore (CBE) and Bangalore (BNGL). Application of GmNPV @ $5x10^7$ POB/ml was effective against the lepidopteran pest complex of cauliflower.

Fungus

Worldwide DBM populations are commonly regulated by three entomopathogens viz., *Beauveria bassiana*, *Paecilomyces fumosoroseus* and *Metarhizium anisopliae* (Metchnikoff) Sorokin. Jadhav Sudhir Damodar et al. (2008) reported that *B. bassiana* was the most virulent against third and fourth instar larvae of *P. xylostella* with LC_{50} values of 1.05×10^5 and 2.74×10^5 spores ml^{-1} respectively. The LC_{50} values were 8.92×10^5, 4.38×10^5, 1.10×10^6 and 2.49×10^6 spores ml^{-1} for the third and fourth instar larvae of the insect for PfCBE and PfPDBC strains of *P. fumosoroseus* respectively. Insecticides tested for compatibility with *B. bassiana* and *P. fumosoroseus* showed that all the insecticides were very toxic at the higher dose. Chlorpyriphos and cartap hydrochloride were compatible only at the lower dose whereas indoxacarb and chlorfenapyr were compatible at both the reduced and recommended doses. The radial growth, sporulation and biomass production of both the fungi were severely affected by insecticides at higher doses.

Insect growth regulators (IGRs)

IGRs cause blockage, disruption or inhibition of any of the events from biosynthesis, storage, release, transport and reception to disturb behavioral or physiological activities which may ultimately prove lethal to insects. The IGR cascade @ 40 g a.i./ha was found to be significantly superior to remaining treatments *viz.,* fenvelerate, delfin, padan in reducing infestation of DBM at 3.7 and 10 days after application. Sangareddy et al. (1999) found novaluron at 0.5, 0.075 and 0.1% to be highly toxic and best to DBM, based on % reduction of larval population in cauliflower. Only two sprays of novaluron were found to be a good alternative for 8-15 sprays of insecticides with better benefit cost ratio (Kulkarni 2001). Harishkumar et al. (2003) reported novaluron @ 0.75 ml/l to provide 90% mortality of DBM larvae under laboratory condition.

Botanical insecticides

Botanicals affect the colonization and feeding of DBM. Weekly dusting of one part of finely ground derris root with nine parts of talc gave good control of *P. xylostella*. Synergists Sesamex and Piprotol improved threefold the effectiveness of neem kernel extract against *P. xylostella*. Piperonyl butoxide enhanced the effectiveness of enriched neem seed extracts, escalating the mortality of DBM larvae. In a field experiment with botanical pesticides on cauliflower, neem oil 2% was far superior to other plant products in overall effectiveness against DBM as well as in increasing the yield (Kalyanasundaram 1995) (Table 6).

Table 6. Efficacy of botanicals on DBM after two applications

Treatment	Dose (%)	No. of larvae per plant before treatment	Reduction in larval population (%)			
			Days after spraying			
			3	7	14	Mean
Neem oil	2.0	17.40	42.8	73.29	73.78	63.29
NSKE	5.0	18.12	54.91	73.94	67.10	65.32
A. calamus FS	2.0	22.61	54.41	72.26	66.45	64.38
Palmrosa oil	3.0	19.98	49.8	64.11	56.36	56.76
V. negundo FS	5.0	22.37	40.65	60.71	49.81	50.39
Illuppai oil	6.0	22.30	29.95	50.36	37.85	39.39
I. carnea FS	5.0	23.46	23.73	42.98	29.64	32.12
Neem leaf extract	5.0	21.52	13.55	29.08	22.46	21.70
P. juliflora FS	5.0	22.16	11.05	23.19	16.50	16.92
Untreated check		25.61	04.48	15.18	09.75	09.81

Table 7. Effect of IPM practices on DBM infestation and cauliflower yield

Treatment	Number of larvae/plant on DAP						Mean	Yield (t/ac)
	35	42	49	56	63	70		
IPM plot	7.1	4.6	4.9	3.2	1.5	1.2	3.75	8.56
Non-IPM plot (Farmers' method)	6.5	5.6	5.7	9.5	9.2	11.6	8.01	5.8
Difference	+0.6	-1.0	-1.8	-4.3	-9.7	-13.4		+2.76
't' value	NS	7.52**	3.48*	14.33**	24.05**	19.84**		11.46**

NS- Non-significant; * - Significant at 5%; ** - Highly Significant at 1%

Table 8. Effect of IPM practices on parasitism by *C. plutellae*

Treatment	Number of larvae/plant on DAP						Mean
	35	42	49	56	63	70	
IPM plot	8.5	12.6	14.6	16.1	10.6	11.4	12.3
Non-IPM plot (Farmers' method)	7.8	4.1	0.4	2.6	1.3	0.8	2.13
Difference	+0.7	+8.5	+14.2	+13.5	+9.3	+10.6	
't' value	NS	10.77**	36.77**	31.46**	27.78**	29.68**	

NS- Non-significant; * - Significant at 5%; ** - Highly Significant at 1%

CHEMICAL INSECTICIDES

Peter et al. (2000) reported spinosad 2.5 SC @ 15, 20 and 25 g a.i./ha to be effective against DBM. The results indicated the superiority of spinosad @ 15 g a.i./ha for better control for a period of ten days with better yield of marketable cabbage heads. Out of two insecticides of microbial origin, abamectin was found to be most effective followed by spinosad. Bioefficacy of avermectin (Vertimec) against DBM in comparison with conventional insecticides revealed that vertimec 1.8 EC @ 15 g a.i./ha was found to be highly effective in checking DBM larval population and also recorded significantly higher yield (Murugan and Ramachandran, 2000). Indoxacarb was as effective as spinosad and significantly more effective than emamectin benzoate. Field experiments conducted to evaluate the bioefficacy of emamectin benzoate (5% SG) recorded that the chemical @ 150 g and 200 g/ha were found to be effective in reducing the larvae and increasing the yield of cabbage (Kumar and Devappa 2006).

Insecticide resistance monitoring

Monitoring studies at monthly intervals in Coimbatore with the available discriminating dose of different insecticides by leaf disc bioassay method revealed that high frequency of resistance was noticed in fenvalerate (92.01%), monocrotophos (90.41%), quinalphos (83.39%), and carbosulfan (80.09%) and moderate level spinosad (43.72%) and emamectin (39.12%). At Ottanchatram, the highest level of resistance was against quinalphos with a mean of 95.81% followed by fenvalerate (93.59%), monocrotophos (80.06%) and carbosulfans (85.39%). Spinosad and emamectin showed moderate resistance level of 34.33 and 53.91%, respectively. The highest level of resistance in Udhagamandalam was against fenvalerate with the mean of 97.04% followed by quinalphos (96.39%), monocrotophos (87.61%) carbosulfan (86.02%), emamectin (49.63%) and spinosad (22.43%).

The specific activities of enzymes in the resistant larvae were estimated at different intervals after the application of insecticides. It was found that there was a high increase in maximum mixed function oxidases (MFO) enzyme activity in Coimbatore, Ottanchatram and Udhagamandalam populations due to exposure of insecticides. However, MFO activity was maximum in the Ottanchatram population (98.72%) at 12 h after insecticidal treatment than in the Coimbatore and Udhagamandalam populations. The MFO activity with carbosulfan ranged from 149.76 to 449.28 in Coimbatore population, 342.31 to 624.01 in Ottanchatram and 599.05 to 798.73 in Udhagamandalam (Muralidharan 2008).

Integrated Pest Management (IPM)

IPM practices showed greater impact not only on the larval population of DBM but also on the parasitism by *C. plutellae*. The DBM larval population was significantly lower (3.75/plant) in the IPM plots than in the non-IPM plots (8.01 larvae/plant). The larval parasitism by *C. plutellae* was also significantly more on IPM cauliflower than on non-IPM cauliflower (Table 7 and 8). Accordingly, the cauliflower yield was higher in IPM plots by 2.76 t/ha than in non-IPM plots (Kalyanasundaram 1995).

IPM practices by farmers

The IPM strategies adopted by growers in Tamil Nadu are: (Fig. 2).

- Growing two rows of mustard after every 25 rows of cabbage as a trap crop at the time of planting. This traps 80-90% of DBM population and other pests. One row of mustard is sown 15 days before cabbage planting and the second row 25 days after planting of cabbage. The first and last row of plots are also mustard.
- Mustard is sprayed with Dichlorovos 0.1% as soon as it germinates.
- Installation of light traps for adult DBM @ 3 traps/acre.
- Release of egg parasitoid *Trichogramma chilonis* at 0.5 lakh/ha 3-4 times at weekly interval on 35 days after planting (DAP) after noticing the moth activity.
- Spraying with NSKE 5% after primordial stage with thorough coverage of the entire plant surface (42 DAP- head initiation stage - most critical stage).
- Release of 2nd instar grub of *Chrysoperla carnea* Stainton @ 1 lakh/ha.
- II spraying with *Bacillus thuringiensis* 1g/lit at 56 DAP (ETL 2 larvae/plant) and repeating it at 10-15 days.
- Release of larval parasite *Diadegma semiclausum* (Ichneumonidae: Hymenoptera) at 50,000/ha, 63 DAP.
- Spraying of indaxocarb @ 29 g a.i./ha on 70 DAP.

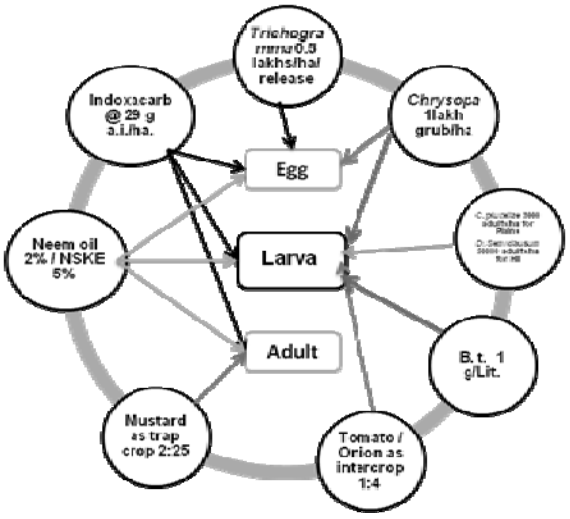

Figure 2. IPM Strategies for DBM

IPM – barriers to adoption

Certain barriers hinder the large-scale adoption of existing and available technologies. These include:

- Farmers' lack of knowledge

- Poor quality seeds and inputs
- Timely availability of biocontrol agents
- Socioeconomic factors

Concerted efforts are necessary for adoption, implementation, and to reduce the pesticide load in the ecosystem.

IPM – future approaches

Present adoption of IPM technologies among farmers of this region is very much scattered. A package that could substantially reduce pest incidence and the use of broad spectrum chemical pesticides is warranted. Certain inputs such as biological control agents *viz*., parasitoids and pathogens, are not available at the doorsteps of farmers. Therefore, it would be appropriate if focus is given on the following:

1) Research priorities

Concerted efforts must be taken to develop cultivars with built-in resistance to DBM and other pests. Even partial resistance would go a long way toward reducing the pest load in this crop. The approach to evolve resistant cultivars could be through both conventional breeding and transgenic approaches. The CIMAA is a classic example. Public perception of genetically modified organisms is changing and people are willing to accept this as a durable technology that is free of pesticides residue. Greater effort is required in this direction through public-private participation.

2) Extension efforts to sensitize farmers and public

Current approaches in extension are not demand-driven or market-oriented. We need to focus in a targeted manner on adoption of IPM by small, marginal, resource-poor farmers. The supply chain and input agencies should develop a mechanism for supply of quality seeds, fertilizers, pesticides, biological control agents, plant products, etc. Farmers need to be educated on these aspects.

On the end users' side, the approach should be for pesticide-free, cleaner products. Consumer preference should determine the need for green technologies. The market forces and supply chain need to be developed on a scientific basis keeping cost under consideration. However, one needs to compromise on seasonal fluctuations in supply and the retail market price. It is here that the role of Uzhavar Sandhais (farmers' markets) established by Government of Tamil Nadu needs to be appreciated; the middlemen have no role to play in this system. The transactions are directly done by the producer and consumer. This is a successful venture now spreading in other states in India.

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Implementation of IPM: experiences with the brassica pest management program against the diamondback moth in Malaysia

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ABSTRACT
The IPM program for the diamondback moth (DBM), *Plutella xylostella*, on brassicas was amongst the most successful developed and implemented in Malaysia. In recent years, doubts on the sustainability of the IPM program have emerged, as evidenced by increasing pesticide misuse by growers. Associated with this is concern about the increasing pesticide load on key natural enemies of *P. xylostella*, such as *Cotesia plutellae, Diadegma semiclausum*, and *Diadromus collaris*. The IPM-DBM program was revisited and this paper reports results of a recent field survey on various aspects of farmer practices and management of DBM in Cameron Highlands. The study revealed that the status of these key natural enemies were still unaffected by the current rampant use of pesticides by farmers. It was encouraging to see that IPM practices and adoption remained unchanged. However, insecticide use is increasing in pest management, with frequent sprays and use of insecticide mixtures, despite the presence of other non-insecticidal options. This paper underscores the issues related to the stagnating adoption of IPM, especially the farmer's perception in the socioeconomic context. Suggestions are provided towards increasing the sustainability of the IPM program in the future.

Keywords
Diamondback moth, IPM, brassicas, adoption, sustainability

INTRODUCTION
The diamondback moth (DBM) was first recorded in Malaysia in 1925 (Ho 1965). It became an important pest in 1941, causing damage to brassicas, such as head cabbage *(Brassica oleracea* var. *oleracea*). Insecticide resistance was confirmed and quantified in 1978 (Sudderuddin and Kok 1978) and gradually the pest became resistant to all major groups of pesticides, including *Bacillus thuringiensis*-based products (Ooi 1986; Syed 1992). More recently, laboratory bioassays using freshly-collected *P. xylostella* from the Cameron Highlands (CH1 strain) indicated significant levels of multi-resistance to spinosad, abamectin, fipronil, and a range of *B. thuringiensis* products (e.g. Cry1Ac, Btk, Cry1Ca and Bta) (Sayyed et al. 2004).

The initiation of an integrated pest management (IPM) program in the early 1980s was triggered by a report in the local media highlighting consumers' health concerns due to farmers' overdosing highland vegetables, especially head cabbage, with pesticides. Farmers were invariably faced with a 'pesticide syndrome' scenario: application of higher doses, increasing frequencies of application, use of cocktails and mixtures, and frequent change of pesticides (Ooi 1997).

IPM program for brassicas
The brassica IPM program in Malaysia evolved over two decades - from early 1980s to the end of the 1990s. It focused on highland cabbage as the target crop as it was a key host of the DBM and encountered the pesticide-associated problems described above. The key pests and disease problems in cabbage are shown in Table 1. Based on our experiences from the plantation sector, viz., oil palm and cocoa, an ecological approach was proposed. The key component in this approach was the use of biological control agents, viz. parasitoids. This was facilitated by the discovery of *Cotesia plutellae (Apanteles)* in 1975 (Lim and Ko 1975) and enhanced through the introduction and establishment of additional parasitoids from overseas, *viz*., a larval parasitoid, *Diadegma semiclausum* and a pupal parasitoid, *Diadromus collaris*. In addition, the development and formulation of the IPM program was underpinned by several studies that included crop and life table analysis and dynamics of key pests, impact assessments of prey-natural enemies and their food sources, development of bio-based components (e.g. trap crop, pheromone and yellow traps, intercropping, etc.) and formulation of economic threshold levels (Lim 1982, Lim et al. 1986, Loke et al. 1992, Ooi and Lim 1989, Sivapragasam 1994, Sivapragasam and Loke 1996, Jusoh et al. 1997, Loke et al. 1997).

Table 1: Keys pests and diseases of cabbage in Malaysia.

Highlands	***Lowlands***
Insects	
• Diamondback moth, *Plutella xylostella*	• Diamondback moth, *Plutella xylostella*
• Black cutworm, *Agrotis ypsilon*	• Cabbage webworm, *Hellula undalis*
• Cabbage head caterpillar, *Crocidolomia binotalis*	• Cabbage head caterpillar, *Crocidolomia binotalis*
• Aphids	• Aphids

• Armyworm, *Spodoptera litura*	• Armyworm, *Spodoptera litura*
	• Flea beetles, *Phyllotreta* spp.
Diseases and others	
• Club root, *Plasmodiophora brassicae*	• Soft rot, *Erwinia carotovora*
• Black rot, *Xanthomonas campestris* pv. *campestris*	• Snails and slugs
• Soft rot, *Erwinia carotovora*	
• Snails and slugs	

The DBM is a major pest in both the highlands and lowlands. Two other key pests confined to the lowlands that can cause significant damage are the cabbage webworm, *Hellula undalis*, and flea beetles, *Phyllotreta* spp. (Table 1). The disease common to both highlands and lowlands is soft rot caused by *Erwinia carotovora*. The other diseases, such as club root and black rot, are confined to the highlands.

The IPM technology package was not a static one but underwent a dynamic process of gradual improvement built on increasing information availability and the specific needs of the situation in the field— similar to a 'box of tools' approach. These entailed refinements in the economic threshold levels (ETLs) with crop age and/or utilizing parasitization levels as criteria for use of pesticides, viz., *Bacillus thuringiensis* (soft approach) versus inorganic pesticides (hard approach) (Sivapragasam et al. 1985, Loke et al. 1992, Loke et al. 1997). The adoption level of the IPM program by farmers varied from about 10% in the late 1980s to a peak of 60% in the mid-1990s (Sivapragasam 2004). The major reasons for adopting the IPM program were: 1) reduction in damage, 2) mitigated or reduced risks due to pests (i.e. DBM) and 3) financial gain based on the superior benefit-cost ratio. Farmers, however, generally reported low favorability to the ease of use of the IPM program.

Revisiting the brassica IPM program

It has been more than 10 years since the last study was done in 2001 on the status of the brassica IPM program in Cameron Highlands. Essentially, the interest is on monitoring the status and thus sustainability of the IPM program. Broadly speaking, sustainability has always been a major issue in many IPM programs. Although the Malaysian program was initially funded by the government, it currently poses challenges due to discontinued funding and changes in the perception of farmers as a result of the changing dynamics of the agricultural landscape such as increasing costs of inputs

especially pesticides. Exacerbated by the weak government extension services and the 'pull' factor (e.g. promotional campaigns) by the pesticide industry, there has been a gradual reversion from an IPM-based to a pesticide-dominated scenario. This is evidenced by the increasing reports on pesticide misuse by growers including claims of adulterations and illegal pesticide use. Our major concern has been on the status of the parasitoids in the field - besides understanding the farmers' perceptions of IPM in the current context. Against this backdrop of concerns, we did a brief study on the current situation in selected brassica farms, focusing on the farmers and the pest management practices in Cameron Highlands. Cabbage farms in each of the three zones, viz., Northern zone (1000 – 1100 m above sea level), Central zone (1400 – 1500 m above seas level and Southern zone (1000 – 1100 m above sea level), were surveyed. During the study, the following parameters were recorded: (i) farm characteristics and general practices; (ii) management of pests and diseases; (iii) insecticide used against DBM; (iv) use of non-insecticide approaches and (v) farmer's practice or adoption of IPM. Comments were also obtained from the farmers to elicit their perception on the sustainability of the current management practice and their specific needs for the future.

The summarized results, shown in Tables 2, 3, 4 and 5, provide a general overview of the current situation in Cameron Highlands.

Farm characteristics, problems and control measures used

Irrespective of the zone, in most farms, DBM is still a major problem in Cameron Highlands, in addition to black rot (Table 2). Although the DBM numbers were relatively low per plant (Table 3), nevertheless, insecticides use still dominated the system. However, it is encouraging to find that the other non-chemical based control measures, such as cultural practices, crop rotation and yellow sticky traps (confined to central and southern zones), had become accepted practices by farmers. Adult sex pheromones, which were once very popular, were not used in any of the zones, possibly due to their high costs.

Table 2. General practices of farmers in Cameron Highlands

Farmer practices/zone	Northern	Central	Southern
Farm type	Conventional	Conventional	Conventional
Main crops	Cabbage, tomato, brinjal	Cabbage, tomato, leeks, lettuce	Cabbage, lettuce, coriander
Cultivation system	Open (70%), rain shelter (30%)	Open (70%), rain shelter (30%)	Open (80%) and rain shelter (20%)
Major problems	DBM	DBM	Black rot, DBM
Control measures (values in parenthesis show the % farmers using the control measure)	Insecticides (100%), *Bacillus thuringiensis* (Bt) (66%), cultural practices (100%), crop rotation (100%); No yellow sticky trap or sex pheromones	Insecticides (100%), *Bacillus thuringiensis* (Bt) (66%), cultural practices (100%); crop rotation (100%); yellow sticky trap (66%); (no sex pheromones)	Insecticides (100%), *Bacillus thuringiensis* (Bt) (33%), cultural practices (100%), crop rotation (100%); yellow sticky trap (33%); (no sex pheromones)

Incidence of parasitoids

All three major parasitoids were still present in Cameron Highlands (Table 3), despite significant insecticide use by farmers (Table 4). The pupal parasitoid *D. collaris* was, however, not recorded in the Southern zone. The major parasitoid in the Northern and Southern zones was *C. plutellae* (Table 3). This was also reported by Syed et al (1997). However, lower numbers of *D. semiclausum* were recorded in the Central zone, contrary to earlier findings by Syed et al. (1997). The reasons for the lower numbers of the latter requires further investigation.

Table 3: Diamondback moth and its parasitoids in Cameron Highlands in 2011 (/month/30 plants)

Zone/host-parasitoids	DBM larva/pupa	Parasitoids		
		Diadegma semiclausum	*Cotesia plutellae*	*Diadromus collaris*
Northern	47.5	28.5	38.5	6.0
Central	34.5	13.5	3.0	4.5
Southern	28.5	12.5	16.0	0.0

Insecticide delivery and use

Table 4 shows insecticide use patterns of farmers, their major source of information, and the criteria used for making a spray decision. Obviously, the current spray numbers per crop and their frequencies were high, somewhat similar to the pre-IPM period, despite the farmer's use of other control measures as indicated in Table 2. A range of ten chemical insecticides and two formulations of *B. thuringiensis*, viz., *aizawai* and *kurstaki*, were generally used as mixtures. The major chemical insecticides used by farmers in Cameron Highlands were chlorantraniliprole, spinosad, and indoxacarb. Spray decisions were based on damage.

Table 4: Insecticide use by farmers in Cameron Highlands.

Insecticide delivery/zone	Northern	Central	Southern
Frequency of use	7 days, 4-6 x per crop	7 days, 4-6 x per crop	7 days, 5-6 x per crop
Type of sprays	Mixtures (insecticides, fungicides)	Mixtures (insecticides, fungicides)	Mixtures (insecticides, fungicides)
Information source	Pesticide Retailers	Pesticide Retailers	Pesticide Retailers
Criterion for spray decision	Damage-based	Damage-based	Damage-based

Adoption and perception

Table 5 shows the factors related to IPM, such as awareness on parasitoids, and the perception of the farmers on DBM and the issues affecting the future livelihood of the farmers. Adoption of IPM still remained at a medium level, similar to that indicated earlier by Sivapragasam (2004). The results also suggested that there are other pertinent factors, such as price fluctuations and general high input costs (including pesticides) affecting the livelihood of the farmers. Thus, the socioeconomic milieu has to be considered to better understand farmer responses toward IPM.

Table 5: Awareness on parasitoids, adoption of IPM and perceptions of farmers in Cameron Highlands.

Factor/Zone	Northern	Central	Southern
Awareness on parasitoids	Yes	Yes	Yes
IPM level*	Medium	Medium	Medium
Perception on DBM	Manageable with pesticides and parasitoids	Manageable with pesticides	Manageable with pesticides
Issues affecting future livelihood	Pesticide pollution in water, instability of vegetable prices and increasing input costs	Pesticide pollution in water, instability of vegetable prices and increasing input costs	Pesticide pollution in water, instability of vegetable prices and increasing input costs

***** *Based on USDA standards (Benbrook et al, 1996): Low adoption – scouting and pesticide applications based on thresholds for one type of pests; Medium adoption - Scouting + Threshold plus 1 or 2 additional practices identified as indicative of the IPM approach; High adoption – Scouting and Threshold plus 3 or more additional IPM practices*

CONCLUSION

The Malaysian DBM-based IPM program for brassicas has, through the years, undergone gradual changes. It was considered a relatively successful program. As indicated earlier, the program took several years to develop and had a relatively strong technical basis. However, its implementation and adoption has met with many impediments, partly due to its rather weak socio-economic focus in the context of overall farming activities. The IPM program and its sustainability are now at a crossroads between a pesticide-dominated system versus a holistic one practiced in the past. Much needs to be done to revert back to the latter system. Based on our experiences, the following suggestions could help the cause of IPM:

1) Supporting a strong developmental aspect to promote biological control and other bio-based technologies, as and when necessary.

2) Strengthening linkages between research and extension as this is crucial for a successful implementation of an IPM program. It is also important to have closer linkages between public and private sector initiatives related to pesticide use. Private-sector initiated advisory services may be considered to complement public-sector driven advisory services.

3) Embarking on more farmer training to empower decision making pertaining to understanding the crucifer ecosystem, concepts of natural balance, and prudent pesticide applications.

4) Considering IPM within the context of the overall farming milieu. As suggested by the preliminary survey, there are other critical factors that impact farmer decisions, such as price vagaries of his produce and the risks of increasing costs of production inputs.

5) Ensuring that all farms are Good Agricultural Practice (GAP) compliant and that the farmers are certified before embarking on vegetable (brassica) production. This is a PUSH factor that could be legislated. Adherence to IPM, as a component of GAP, helps promote the adoption of IPM practices and would ensure proper auditing and certification of the farmer (as a qualified grower) and his production system.

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Control of brassica pests – an example of successful IPM in Australia

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ABSTRACT
Plutella xylostella is a serious pest of brassica crops in Australia as it is in many other parts of the world. This includes horticultural crops, such as *Brassica oleracea* cultivars (broccoli, cabbage, cauliflower and Brussels sprouts), broad-acre crops such as *Brassica campestris* (canola) and fodder brassicas such as *Brassica rapa* (turnip). Resistance to insecticides has led to crises in pest control in different industries in recent years including all of the examples listed above in Australia. IPM Technologies P/L has been successful in helping farmers to implement an IPM strategy in each of the different brassica crop types listed. We describe in this paper how the successful implementation in commercial crops was achieved and provide examples. The key to successful adoption was to provide growers with the means to control all pests in a compatible manner, so that the control measures for one pest did not disrupt control of other pests. Selection of appropriate pesticides that can fit with this strategy is a very important component of the strategy that we have developed. To support this information requirement IPM Technologies P/L has conducted its own testing of pesticides on a range of beneficial invertebrates, and some results are presented in this paper to highlight how decision-making is influenced.

Keywords
integrated pest management, brassicas, implementation, insecticides

INTRODUCTION
IPM Technologies Pty Ltd is a private research and consultancy company of entomologists based in Melbourne, Australia (see www.ipmtechnologies.com.au). The company provides advice to farmers and agronomists interested in changing practices and adopting integrated pest management (IPM). Usually this involves a change from a pesticide-based approach to using one based on biological and cultural control options, with selective pesticides used as support rather than the basis of control. Although expert entomological advice is the basis of any assessment, the process of implementing change depends on more than entomological issues and this is something that we have concentrated on in our work (Horne et al., 2008; Page and Horne 2007; Horne and Horrocks, 2010). This has resulted in high levels of adoption of IPM that are unusual worldwide (Ehler 2006). This includes adoption of IPM by brassica growers in Australia who need to deal with pests such as *Plutella xylostella*,(diamondback moth), *Pieris rapae* (cabbage white butterfly) and *Brevicoryne brassicae* (cabbage Aphid).

In Australia, Werribee South is a significant production area for brassicas, accounting for about 17% of the national brassica production (ANRA 2011). IPM Technologies P/L currently works with 15 growers who produce approximately 8,000 t of produce, which amounts to about 30% of brassica production from Werribee South.

In addition to working with brassica farmers in Werribee South, IPM Technologies P/L has also helped to implement IPM on brassica farms in other parts of Australia, including the Tasmanian company, Harvest Moon. Harvest Moon is the largest fresh-market producer of broccoli in Tasmania, planting 400 ha per year for an annual harvest of approximately 3,500 t. Our IPM approach has also been successfully adopted by broad-acre growers, including canola growers, and fodder brassica farmers (Horne and Page 2008).

The difference between the regions from an IPM point of view includes the fact that IPM Technologies P/L can do the monitoring and decision-making on behalf of growers, where we are capable of visiting farms weekly, but in other places we need to offer a different type of support. Both types of support have proved successful but in the long-term, even those growers who we are not able to provide with a full monitoring service are likely to be better off. This is because we can teach growers and agronomists how to assess things within an IPM strategy and this is a lasting effect.

IPM in brassicas
Brassicas are grown around the world and include plants such as cabbages, cauliflowers, broccoli, pak-choi, canola and turnips. So they cover the range from horticulture, broad-acre and forage pastures. However, the pests that occur are mostly the same in all of these (some exceptions with establishment pests of broad-acre crops) and include diamondback moth, cabbage white butterfly and cabbage aphid. In the last few decades diamondback moth has changed from being a minor pest to the most serious pest

of brassicas because it has developed resistance to a vast range of insecticides, and is likely to continue to do so.

Although some have tried to talk of *IPM for diamondback moth control*, in fact what is needed is *IPM for brassicas*, of which diamondback moth is a key pest. Diamondback moth is a massive pest problem in many parts of the world and many research projects have been conducted to try and develop effective control. In our experience, when considered as just another pest, with a range of natural enemies and with cultural controls that can be used, with some insecticides used as support, then control is actually not difficult. We have seen this work in a range of crops across horticulture, broad-acre and fodder crops. The main factor is to use the biological and cultural control options first, and use the least disruptive pesticides as support tools.

The basis of the pesticide support in our program has been BT (*Bacillus thuringiensis*) based product formulations such as *Bt* strain *kurstaki* and *Bt* strain *aizawai* – not because of any greater kill rate compared to other products but because of the minimal impact on beneficial species that help to control all pests. Basically, the use of these products will kill a large number of pest caterpillars and also leave intact the populations of predators and parasitoids that will give far greater control of the pests.

The combination of biological control, supported by selective insecticides is an effective alliance that works to control a range of pests in brassica crops. Cultural controls such as rotation, weed management and sequential planting are also important. The farmer who grows brassicas in recent years would have experienced difficulties with control of diamondback moth in particular, and increased reliance on new insecticides. Changing to reliance on beneficial insects that the farmer may never have seen and BT-based products which have been available for over 50 years is a difficult task to expect farmers to achieve alone.

Adoption of IPM

IPM is promoted by many government and non-government agencies around the world and yet adoption rates are typically low (Horne et al., 2008). We at IPM Technologies P/L have been successful in achieving high levels of adoption with farmer groups and the obvious question is "How did we achieve this?"

Table 1 describes some of the relative advantages and disadvantages of adopting IPM and it is clear that there are often long-term benefits of IPM but short-term benefits of pesticides. Table 2 compares aspects of IPM strategies and a pesticide-based approach to pest management. The range of pests that we have had to deal with in various crops includes insecticide resistant pests such as western flower thrips, two-spotted mites and lettuce aphid; it includes dealing with pests of crops grown for cosmetic appearance or for which their appearance is critical and it includes insects that are vectors of viral diseases (such as barley yellow dwarf virus in cereals, tomato-spotted wilt virus in capsicums and potatoes, and leaf-roll virus in potatoes).

The reason for success is that we have largely operated in the commercial sector and so have been able to implement IPM in a manner more aligned with the left-hand column of Table 2 rather than the right-hand column. That is, from the grower's perspective, their IPM strategy is compact, easily incorporated into regular farming operations and the results of implementations are readily apparent. This is because our ongoing monitoring and consultation shifts the decision-making responsibility from the farmer to us. Therefore, IPM becomes relatively easy to apply which is comparable to their former heavy reliance on insecticide applications.

Table 2 highlights the differences that may be experienced from a farmer's point of view when deciding between a pesticide-based strategy and an IPM strategy. Furthermore, when the starting point for any individual farmer or farm is that a pesticide-based strategy is known, legal and it works, then the decision to adopt something unfamiliar and unproven (on the farmer's own crop) is even more difficult.

Similarly, particular cultural control practices such as rotation may give substantial benefits in the longer term but these benefits will probably not be seen in the life of the current crop. Where the crop is a fairly short-term crop such as many brassica vegetables, then the benefits need to be seen within a matter of days or

Table 1. Advantages and disadvantages of adopting IPM (from Page and Horne, in press)

Advantages	Disadvantages
Reduced dependence on pesticides	More complex than control by pesticide alone and requires a shift in understanding
Increased safety to farm workers, spray operators and the community	Requires a greater understanding of the interactions between pests and beneficials
A slower development of resistance to pesticides	Requires a greater understanding of the effects of chemicals
Reduced contamination of food and the environment	Increased time and resources
Improved crop biodiversity	Level of damage to the crop may initially increase during transition to an IPM programme, in some horticultural crops

Table 2. A Comparison of IPM and Pesticides based supports (derived from Bajwa and Kogan 2003)

Pesticides	IPM
Compact technology	Diffuse technology with multiple components
Easily incorporated into regular farming operations	At times difficult to reconcile with normal farming operations
Promoted by private sector	Promoted by public sector
Aggressive sales promotion supported by professionally developed advertising campaigns	Promoted by extension personnel usually trained as educators not as salespersons
Results of applications usually immediately apparent	Benefits often not apparent in the short term
Consequently: pesticide technology was rapidly adopted	**Consequently: Adoption of IPM technology has been slow**

weeks, if they are to be evident in the life (and costings) of the current crop. Problems may be seen only several crops later and so are more difficult to cost directly within only one crop.

The usual method of achieving adoption of IPM with either individual growers or groups of growers is for IPM Technologies P/L entomologists to provide IPM advice in commercial crops with collaborating farmers. This means that the entomological advice is on trial and will certainly be tested commercially. The test is for entomologists to prove that what they say can work, and be accountable for such advice, and for growers to implement the different type of advice. It is essential that the advice given be site specific and not general or vague advice given to a crop type. That is, the IPM advice must work on the specific farm that is asking for advice, and the farmer must be prepared to implement such advice. Typically, to reduce risk, a farmer will attempt a new approach on one paddock and trial something new. If it works then adoption on larger plantings will follow. Once the IPM advice is valued and proven, then on-going support does not need to be so intense and could be dealing with unforeseen problems from a distance as they arise.

Pesticides and brassica pests

The major pests of brassicas are caterpillars and aphids. The pesticides applied to deal with these pests are often the cause of further problems due to the effects of even the newer and more selective insecticides on beneficial species. Selective insecticides provide valuable pest control options in crops because they are often compatible with the many predatory and parasitic species that have potential to control pest populations. The availability of these insecticides has made an enormous contribution towards the adoption of IPM in Australian crop protection, but knowledge of their impacts on the array of beneficial species that provide biological control of pest species is vital. This is because even these more selective insecticides can cause negative acute or sublethal effects on a range of predatory and parasitoid species (Galvan *et al.* 2005).

We have tested the acute and long-term effects of pesticides on beneficial insects in our laboratory over a number of years. Acute bioassays have measured the impacts of direct wet sprays and dried residues on individuals over 72 h, and long-term tests have examined pesticide impacts over one generation of a population by measuring stage-specific mortality, net reproductive rate, generation time and the intrinsic rate of increase of the population (Cole *et al*, 2010). The data has allowed us to make informed analyses of the likely impacts of particular pesticides on beneficial organisms in the crop.

Several insecticides have been tested for their impacts on beneficial insects that commonly occur in brassica crops in Australia (Table 3).

Table 3. Impacts of selected insecticides on beneficial species that commonly occur in Australian brassica crops. L: low impact, M: moderate impact, H: high impact

Species:	*Mt*	*Ct*	*Nk*	*Mv*	*Tc*	*Ds*
Insecticide						
B. thuringiensis toxins	L	L	L	L	L	L
chlorantraniliprole	L	L	L		L	L
pymetrozine	L	H	L	L	L	L
pirimicarb	L	M	M	L	L	
flubendiamide	L	L	L			L
spinosad	L	L	L	H	H	
indoxacarb	L	H	H		H	
spirotetramat	L	L	L			

Mt: Micromus tasmaniae, Ct: Coccinella transversalis, Nk: Nabis kinbergii, Mv: Melangyna viridiceps, Tc: Trichogramma carverae, Ds: Diadegma semiclausum.

Our approach to implementing IPM has required us to know what effects pesticides have on beneficials and the only way to do this has been to conduct the testing ourselves, to achieve the level of accuracy required. We know that pesticides such as Dipel and XenTari *(B. t. kurstaki* and *B. t. aizawai*, respectively) are safe to beneficial species but do not always give as high a kill-rate as other chemical products. However, we do not need such a high kill rate if there are populations of beneficials that are resident in the crops.

Ongoing research into IPM related issues is of primary importance if the implementation of IPM is to grow, both in Australia and worldwide. Our research over recent years has included observing the impacts of inundative releases of *D. semiclausum* on parasitism levels of diamondback moth larvae and the effects of UV irradiation and rainfall on efficacy of *B. thuringiensis* products.

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Occurrence and control of *Plutella xylostella* (Lepidoptera: Plutellidae) in Yunnan, China

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ABSTRACT
Yunnan province has become one of the major vegetable growing areas in China. The area under vegetable production is about 0.6 million ha and of which, 50% is occupied by cruciferous crops. The diamondback moth (DBM), *Plutella xylostella* (L.) is a serious, and destructive insect pest of cruciferous crops in Yunnan. A number of severe outbreaks have prompted many studies on the ecology and control methods. Our studies show that *P. xylostella* has been controlled by various chemical pesticides in Yunnan, but in recent years, it developed resistance to most of these insecticides especially abamectin, β-cypermethrin, tebufenozide, chlorfluazuron and fipronil. The occurrence and damage of DBM population show distinct specificities. From March to September the population in the field is at the peak. The DBM population in spring and summer is higher than in autumn and winter. *Diadegma semiclausum* Hellen plays a dominant role in the biological control of DBM in Yunnan and its parasitism varies from 10.4%-47.4% in the field. Sex pheromone trap is an effective forecasting tool and control method. The DBM population can be reduced significantly (41.94%-80.00%) by continuously using the sex pheromone traps during the growing season. Other suitable control methods such as cultural control, chemical control and so on were evaluated. IPM was an effective and durable way of controlling DBM and reducing its losses in Yunnan.

Keywords
Plutella xylostella, occurrence, control

INTRODUCTION
Yunnan province, located in the southwest of China, has been become one of the major vegetable growing areas. The area under vegetable production is about 0.6 million ha, and of which over 50% is occupied by cruciferous crops like cabbage, Chinese cabbage, cauliflower and broccoli. The diamondback moth (DBM), *Plutella xylostella* (Lepidoptera: Plutellidae) is a serious pest of cruciferous crops throughout the world (Cheng, 1988; Talekar and Shelton, 1993). It is also a serious and destructive insect pest in vegetable planting areas of Yunnan, and can be found on crucifers throughout the year provided the crop is planted continuously. The extensive use of chemical insecticides has placed *P. xylostella* under pressure of high chemical selection. Insecticides have dominated attempts to control *P. xylostella* for many years following the development of resistance (Tabashnik, 1990; Shelton, 1993; Zhao, 1996). DBM has become one of the most difficult insect pests to be controlled worldwide, and has developed resistance to almost every insecticide applied in the field (Sarfraz and Keddie, 2005; Sayyed, 2005; Attique, 2006). In the IPM strategy of DBM management, using sex pheromone traps (Reddy and Urs, 1997) and release of natural enemies (Momanyi, 2006) are important and economical ways.

In order to provide a basis for developing resistance management strategies, it is necessary to evaluate the resistance of DBM to the commonly used insecticides in the field. However, such an evaluation in Yunnan Province still remains unavailable. Our research reports the population dynamics of DBM, the susceptibility of DBM populations to 11 commonly used insecticides and evaluation of the control effects of sex pheromone traps and *D. semiclausum*, a larval parasitoid on DBM population.

MATERIALS AND METHODS

Population dynamics
Larval populations of *P. xylostella* were surveyed at Lincang (Southwestern), Tonghai (Southern) and Zhaotong (Northeastern) areas of Yunnan at intervals of 5-10 d. All larval instars on 25 cabbage plants in every field, fixed in the planting seasons, were counted. In each locality more than five cabbage fields were surveyed at the same time.

Bioassays
Insecticides: 11 commonly used insecticides *viz.,* 20% diafenthiuron EC, 3% Bt (*Bacillus thuringiensis*) WP, 10% chlorfenapyr EC, 25% spinosad SC, 5% fipronil EC, 98% cartap WP, 10% indoxacarb EC, 5% chlorfluazuron EC, 10% tebufenozide EC, 5% β-cypermethrin EC, 2% abamectin EC were provided by Institute of Plant Protection, Guangdong Academy of Agricultural Sciences, China.

Insects: Approximately 400 DBM pupae were collected in each location from Tonghai county, Midu county and Kunming city, and maintained separately in the laboratory. Adult moths were released into insect cages for mating and laying eggs on aluminum foils treated with cabbage juice. Foils with eggs were transferred to fresh radish seedling for hatching and feeding at 25± 1^0C, 60-70% RH and a photoperiod (16:8; light : dark). Baseline-susceptibility for the selected insecticides was kindly provided by Dr. Yidong Wu (Nanjing Agriculture University, China).

Leaf-dipping method for conducting bioassays on DBM was used (Mohan and Gujar, 2003), A series of concentrations from commercial formulations of selected insecticides were prepared. Fresh Chinese cabbage (*Brassica campestris* L. ssp. Pekinensis) leaf discs of approximately 6.5 cm diameter were cut from cabbage plants grown in the greenhouse, and thoroughly washed with water and then air dried for about 20 min. Leaf discs were dipped into each insecticide solution for 10 sec and air-dried at room temperature for about 1 h. Treated leaf discs were transferred to clean Petri dishes (diameter 7 cm) and 10 DBM larvae (3rd instar) were introduced in each petri dish. Each insecticide had five concentrations with four replications for every concentration. All Petri-dishes were kept in the same condition as mentioned above. Mortality was assessed after 48 h or 96 h depending on the mode of action of an insecticide. The larvae were considered dead if they did not respond when touched with the brush.

Sex pheromone trapping

Volatile sex pheromone products were purchased from New Con Inc., (Ningbo, Zhejiang, China). Sex pheromone traps for controlling DBM were installed in 13.3 ha of vegetable fields during Sep-Nov 2008 in Luliang county (Northeastern of Yunnan). 30 plastic tubes containing the sex pheromone were set up in 1 ha cabbage field, and replaced every month with fresh ones. The adult population of *P. xylostella* was surveyed and counted at intervals of 7 d.

Biological control

The larval parasitoid, *D. semiclausum* was reared in the laboratory at 22±1°C, 60-70% RH and a photoperiod (14:10; light : dark) (Chen, 2003). 30,000 individuals of this insect were released in 1 ha in early May in Tonghai county. Larvae and pupae of DBM and cocoons of parasitoids were collected from the field at intervals of 5-10 d; larvae and pupae were kept separately in glass jars and provided with fresh leaves of cauliflower till pupation. Sick and sluggish larvae were sorted out and kept for parasitoid emergence. Cocoons of parasitoid were also kept separately in jars for their emergence. The emerged adults of parasitoids were then identified.

Statistical analysis

Concentration-mortality data were analyzed by Probit analysis using POLO (LeOra Software, 1997). Mortality was corrected using Abbott's formula (Abbott, 1925) for each Probit analysis. Resistance ratio (RR) were estimated at median lethal concentration (LC_{50}) level as RR = LC_{50} of field population/LC_{50} of susceptible population.

Trapping control effects (%) = [(Densities of DBM adults in control field – Densities of DBM adults in trapping field)/Adult densities in control field)] X 100.

Parasitism (%) = [(Number of parasitoids pupae) / (Number of DBM pupae and parasitoids pupae)] X 100.

RESULTS AND DISCUSSION

Larval population dynamics

The population dynamics of *P. xylostella* varied in the different vegetable planting areas of Yunnan. There is a distinct population peak throughout the year and the occurrence of larvae in spring and summer is more serious than in autumn and winter seasons. In Lincang (Southwestern of Yunnan), the population peak of *P. xylostella* occurred between March - May with the highest larval density of 1.44 larvae/plant on 12th May 2009 and 1.32 larvae/plant on 24th Mar 2010. In Tonghai (Southern), the population of *P. xylostella* reached the highest between May-September with a maximum larval density of 5.12 larvae/plant on 2nd,Jun 2009 and 2.52 larvae/plant on 11th, Aug 2010. In Zhaotong (Northeastern), population peak occurred between May-June with the maximum larval density on 11th May in 2009 (3.6 larvae/plant); but a smaller peak of 0.48 larvae/plant on 28th May in 2010 was observed (Figure 1). It has already been reported that seasonal abundance of *P. xylostella* on cauliflower was significantly affected by temperature, humidity and rainfall as well as parasitoids (Shelton, 2001; Syed, 2003). In the rainy season, larval population of *P. xylostella* decreased and due to the unfavorable conditions for the immature stages (Talekar, 1993; Iga, 1985; Ayalew, 2006), which was also confirmed in the present study. So, the population dynamics of *P. xylostella* in different vegetable planting areas as well as years were different.

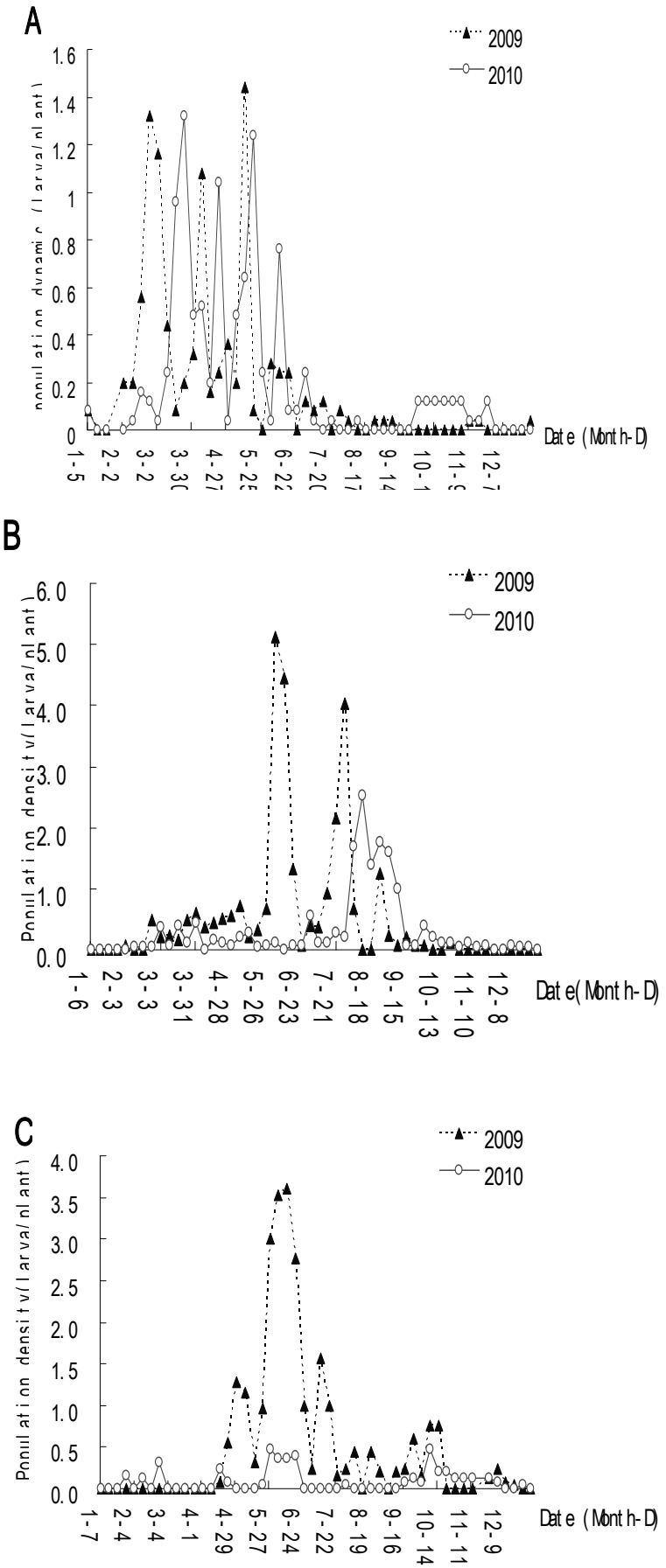

Figure 1. Larvae population dynamic of *Plutella xylostella* in Lincang (A), Tonghai (B), Zhaotong (C) (2009-2010)

Insecticide resistance

The resistance *of P. xylostella* in Tonghai, Midu and Kunming to 11 selected insecticides was determined in spring of 2010. The resistant level of *P. xylostella* to insecticides showed variations in different vegetable growing areas. On the whole, *P. xylostella* was sensitive to diafenthiuron and *B. thuringiensis* in Yunnan (Resistance ratio, RR□10) (You, 2007), high tolerance to β-cypermethrin and abamectin (RR□160), and low to high resistance to other insecticides. The results were listed in Table 1 in detail.

In Tonghai county, the populations of *P. xylostella* exhibited low level resistance (RR<10) to chlorfenapyr, moderate level resistance (RR<40) to spinosad, fipronil, and cartap, and high level resistance (40<RR<160) to indoxacarb and chlorfluazuron, and very high level of resistance (RR>160) to tebufenozide. According to the field population resistance results, *P. xylostella* was susceptible to chorfenapyr, diafenthiuron and *B. thuringiensis*, and hence these chemicals can be recommended as the preferred insecticides to be used to control the resistant DBM population; but they should not be continued for more than two sprayings in a planting season. Spinosad, fipronil and cartap can also be recommended, but they should be confined to one spraying in a planting season.

In Midu county, *P. xylostella* had a moderate level resistance (RR<40) to chlorfenapyr, spinosad, fipronil and cartap, high level of resistance (40<RR<160) to indoxacarb, chlorfluazuron and tebufenozide. *P. xylostella* was susceptible to diafenthiuron and *B. thuringiensis* and these two chemicals can be recommended for the control of resistant population, although they should be confined to only two times in a planting season. Chlorfenapyr, spinosad, fipronil and cartap can also be recommended for a single spraying in a planting season.

In Kuming City, the results showed that *P. xylostella* had low level resistance (RR<10) to chlorfenapyr and fipronil, moderate level resistance (RR<40) to spinosad, cartap, indoxacarb and chlorfluazuron, and high level of resistance (40<RR<160) to tebufenozide. Since *P. xylostella* was susceptible to chlorfenapyr, fipronil, diafenthiuron and *B. thuringiensis*, they can be recommended for the control of resistant population; however they have to be confined to two times in one planting season. Spinosad, cartap, indoxacarb and chlorfluazuron can also be recommended for a single spraying in one planting season.

Table 1. Resistance ratio of different insecticides to larvae of *P. xylostella* in Yunnan (Spring, 2010)

Insecticides	Resistance Ratio		
	Tonghai County	Midu County	Kunming City
Diafenthiuron	0.86	1.60	2.20
BT	4.50	3.30	5.60
Chlorfenapyr	8.71	16.30	3.10
Spinosad	16.81	25.60	27.0
Fipronil	20.77	21.21	6.20
Cartap	39.34	22.0	11.40
Indoxacarb	57.50	66.90	30.10
Chlorfluazuron	62.10	53.50	12.90
Tebufenozide	302.14	64.30	45.10
β-cypermethrin	528.91	196.60	283.50
Abamectin	1415.05	987.25	977.30

Sex pheromone trapping control

P. xylostella population was reduced by 41.94 - 80.00% after using the sex pheromone traps (Table 2). The sex pheromone traps installed at the rate of 30-45 traps/ha were effective for controlling DBM in the field, and could reduce 30% of insecticides per planting season and save 900 RMB/ha in terms of control costs.

Earlier studies reported that field trapping experiments suggested that a red rubber septum impregnated with synthetic sex pheromone (a mixture of Z11-16:Ald, Z11-16:Ac and Z11-16:OH at a ratio of 7:3:1) could catch 98.3 male moth from May to July in Songjiang (Shanghai, China) (Dai *et al*. 2008). A sex pheromone trap caught approximately 30 male moths in a day in Suwon (Korea) (Yang *et al*. 2007). The total number of moths caught during the crop season was more than 1600 per trap in selected locations of Karnataka, India (Reddy and Urs 1997). In the field, the higher sex ratio of (female to male) may lead to lower number of eggs laid, and lower hatchability of eggs (Gong, 2010). Thus, trapping of male moths of DBM could reduce the subsequent population build-up.

Table 2 Control effects of sex pheromone traps on *P. xylostella* (Luliang, 2008)

Recorded Date	Mean Population Density (Larva/Plant)		Control Effect (%)
	Sex Pheromone	CK	
23 Sep	0.48±0.01	1.25±0.08	61.60±4.23
30 Sep	0.35±0.02	0.73±0.04	52.05±2.68
7 Oct	0.28±0.05	0.52±0.01	46.15±1.92
14 Oct	0.18±0.03	0.31±0.05	41.94±3.11
21 Oct	0.08±0.01	0.33±0.04	75.76±2.12
28 Oct	0.10±0.02	0.26±0.02	61.54±3.75
4 Nov	0.04±0.01	0.20±0.03	80.00±2.34
14 Nov	0.04±0.01	0.18±0.04	77.78±5.06

Diadegma semiclausum

D. semiclausum, introduced from Taiwan to Yunnan in 1997, has played a dominant role in the biological control of the DBM (Chen 2003). In 2009, parasitism was found to increase from May to September (4-45%) (Table 3). The wasp parasitism can go over 45% in August and September in the fields when *B. thuringiensis* sprays are combined with cultural methods to protect the wasp and improve the level of parasitoid. For example, in Philippines the combination of *D. semiclausum* releases and BT applications has reduced the insecticide use at least 50-70% (Amend and Basedow, 1997). In Japan, parasitism by *D. semiclausum* reached 53% in the release field (Noda, 2000). In Kenya, seven months after release *D. semiclausum*, parasitism reached to a maximum of 60% (Momanyi, 2006).

Table 3. The parasitism of *D. semiclausum* on DBM in the field of Tonghai (2009)

Date	Adults		Parasitism (%)
	P.xylostella	*D. semiclausum*	
19 May	153.4±5.6	15.9±2.6	10.4±1.8
15 June	99.6±4.7	22.4±3.4	22.5±2.4
15 July	75.8±6.2	27.9±2.9	36.8±3.2
17 Aug	86.6±4.1	41.1±5.2	47.4±4.1
21 Sep	117.0±9.3	52.7±4.8	45.0±3.7

CONCLUSION

The *P. xylostella* is still a serious and destructive insect pest of cruciferous crops in vegetable planting areas of Yunnan. The population dynamics of *P. xylostella* varied in different vegetable planting areas and years. Larvae density of Tonghai county was the highest compared with the other areas in Yunnan and had a longer population peak from May to September. Larval population peak of Lingchang and Zhaotong was only in March-May and May-June, respectively. The resistance of *P. xylostella* to 11 selected insecticides was determined in 2010. *P. xylostella* developed resistance to most insecticides like abamectin, β-cypermethrin, tebufenozide, chlorfluazuron and fipronil in Yunnan in recent years. The resistant level of *P. xylostella* to insecticides showed variations in the different vegetable growing areas of Yunnan. *P. xylostella* showed susceptibility to diafenthiuron and *B. thuringiensis*. These two insecticides can be recommended as the preferred insecticides for the control of resistant population. Chlorfenapyr, spinosad, fipronil, indoxacarb and chlorfluazuron can also be recommended. β-cypermethrin and abamectin should be prohibited for the control of DBM in the field. *D. semiclausum* plays a dominant role in the biological control of *P. xylostella* in Yunnan; its parasitism can reach 47.4% during May to September in the field when released. Sex pheromone trap is an effective forecasting tool and control method, too. The population of *P. xylostella* can be reduced by (41.94-80.00%) by continuously using sex pheromone traps in a growing season. Thus, IPM is an effective and durable way of controlling *P. xylostella* and reducing losses in Yunnan. Many suitable control methods like cultural control, sex pheromone traps, parasitoid release and insecticide use should be integrated for controlling the *P. xylostella*.

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Diamondback moth (*Plutella xylostella)* management in Lao PDR

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ABSTRACT

Agriculture is the most important section in Lao PDR. Cabbage is one of the most important vegetables in Lao PDR, and produced both for local consumption and export. Insect pests are a serious production constraint and the important insect pests on cabbage are the foliar feeding insects such as *Plutella xylostella*, *Spodoptera litura* and striped flea beetle *(Phyllotreta Striolata*). Hence farmers use chemical pesticides to control them; but usage of pesticides affect the human and environmental health. Presently, the Ministry of Agriculture and Forestry of Lao PDR is promoting Clean Agriculture policy by continuing support and improve Good Agriculture Practices, Production Free Pesticides, Organic Agriculture and conventional Agriculture, which are potential sources in agricultural production of Lao PDR. Usage of biological control and botanical extracts which support directly to clean agriculture policy and this is a way to reduce the use of chemical pesticides in agriculture. At present, the diamondback moth (DBM) management strategies are being developed and promoted in Lao PDR through conducting field trials, demonstration plots and training on the production and use of botanical pesticides, and natural enemies conservation in field such as parasitoids, , predators (ladybird beetle, spiders), etc for technical staffs and farmers. Trials, demonstrations and trainings are conducted in Vientiane capital, Vientiane province and Champasack province that grow more cabbages.

Keywords

Diamondback moth, Lao PDR, biological control, demonstrations, training

INTRODUCTION

Lao People's Democratic Republic (Lao PDR) is a small landlocked country which is enriched by diverse ecological characters and the national economy is mainly based on natural resources, especially agro-biodiversity that basically contributes to livelihood of the Lao people who live among the poorest in the world. Agriculture is the most important section in Lao PDR. Crop production is largely dominated by paddy rice followed by other important crops including maize, job-tear, coffee, beans, cabbage and fruit trees. Insect pests are serious production constraints in most crops. Although integrated pest management strategies are being promoted by various agencies including Food and Agriculture Organization (FAO) of United Nations, growers still mainly rely on chemical pesticides to manage the noxious pests. Information and management on bio-pesticide application is lacking, which is essential for the sustainable development and many factors have not yet been focused.

Cabbage is an important vegetable crop, which is produced both for local consumption and export. The farmers always face problems with insect pests. *Aulacophora* sp., *Epilachna* sp., *Eurydema pulchrum, Helicoverpa armigera*, *Hellula undalis, Lipaphis erysimi*, *Phyllotreta striolata*, *Pieris rapae*, *Plutella xylostella*, *Spodoptera exigua*, *S. litura*, *Thysanoplusia orichalcea* and *Trichoplusia ni* were identified as the insect fauna that feed on cabbages in a field survey during 2009-2010. However, the important insect pests on cabbage in Lao PDR are the foliage feeders such as *Plutella xylostella*, *Spodoptera litura* and striped flea beetle *(Phyllotreta Striolata*).Hence farmers use chemical pesticides to control them; but usage of pesticides affects the human and environmental health.

Diadegma semiclausum, a larval parasitoid of DBM was introduced to many Southeast Asian countries including Lao PDR, and controlled the DBM effectively in the highlands (Rowell et al., 2005). The Lao PDR joined the FAO Regional Integrated Pest Management program in 1996 and started its first rice Farmer Field School training in 1997, which was later expanded to various crops in several provinces. Through this program, *D. semiclausum* and an indigenous parasitoid, *Cotesia* sp., which attack DBM, were introduced to southern provinces (Boloven Plateau) (Anonymous, 2010).

Presently, the Ministry of Agriculture and Forestry of Lao PDR is promoting Clean Agriculture policy by continuing support and improve Good Agriculture Practices, Production Free Pesticides, Organic Agriculture and conventional Agriculture, which are potential sources in agriculture production of Lao PDR. The Plant Protection Centre (PPC), Department of Agriculture, Lao PDR has recently focused on developing biological control options as alternatives to chemical pesticides as well as offering training courses to farmers on biological control. Hence, this paper briefs some of our recent attempts in managing the DBM on brassica vegetables in Lao PDR.

Trial on effectiveness of *Bacillus thuringiensis* (Bt), neem extract and insecticide to control DBM

A field trial was conducted with *Bacillus thuringiensis* (Bt), neem extract and chemical insecticide (cypermethrin, Secsaigon®) to manage the DBM. The plot size was 3-m X 2.5-m, with about 60 plants/plot. To prevent the effects of spray drift, block to block and plot to plot distance was 1.5-m. The first application was made on 15 days after planting and weekly application was repeated thereafter until harvest. The spray volume was 400 liter/ha. Data were collected one day before and

three days after each spray application. Direct counting on larvae, pupa and adult of DBM and its natural enemies was performed in ten randomly selected plants per plot.

After the first application, Bt and neem reduced the numbers of *P. xylostella*. However, the chemical pesticide (cypermethrin, Secsaigon®) was highly effective than the bio-pesticides. The subsequent applications were also found effective throughout the growing season. Bt was proven moderately effective against *P. xylostella*. However, neem was less effective in controlling this insect. This could be due to the difference in the quality of the product. Both Bt and neem had less effects on natural enemies such as *D. semiclausum*, although chemical insecticide (Secsaigon®) reduced the population of *D. semiclausum*. Thus, the effectiveness of bio-pesticides against the target pests and their compatibilities with the natural enemies compared to the farmers' practice were demonstrated to the farmers.

Training on production and use of botanical pesticides, and conservation of natural enemies

The training was organized in several villages in Vientiane province, Vientiane Capital and Champasak province and about 30-35 farmers participated in each village for two days. In the beginning of the training, the questions such as why they apply pesticides were raised to the farmers to share their experience. This was followed by a briefing on the effects of pesticides on pests, natural enemies and human-beings. They were then explained about agro-ecosystem analysis and insect classification or grouping. Biology and ecology of diamondback moth (DBM), *Plutella xylostella*, flea beetle (*Phyllotreta striolata*), cabbage webworm (*Hellula undalis*) and cabbage white butterfly (*Pieris* sp.) were taught to the participants. Since the farmers are unaware of the availability of other pest management options, alternative strategies using biological control agents, bio-pesticides, botanical pesticides and conservation of natural enemies were introduced. The basic concept of biological control such as what are bio-control agents-, why they are important- and how do they work against insect pests- was explained to the participating farmers. Commonly available parasitoids and predators in cabbage fields, including *Diadegma semiclasum*, *Cotesia* spp., spiders and ladybird beetles were shown to them. The mode of action of bio-pesticides such as *Bacillus thuringiensis* (Bt) and the method to use it were also briefed. The production and field application methods for three botanical pesticides including neem, garlic and tobacco extracts were demonstrated to the farmers. Finally, the uptake and utilization of systemic insecticides by the plants against sucking insects were taught, since they may not interrupt the biological control agents that target the foliage feeding lepidopterans and coleopterans.

This training focused on learning by doing, which is easy to understand for the growers. The main intention of the training was to raise the awareness of the farmers on organic farming, good agricultural practices (GAP) and integrated pest management (IPM) concepts. Although the farmers understood the effects of chemical pesticides on health and environment, they still believe that chemical pesticides is the best way to solve their pest problems and do not concern about pesticide residues in plant produces. The farmers are very much interested in using biological control agents and botanical pesticides, and they should be made available for proper adoption.

CONCLUSION

The results from efficacy experiment revealed that Secsaigon® (cypermethrin) provided the best control of *P. xylostella* on cabbage than Bt. Population of *P. xylostella* in Bt treated plot was moderately suppressed as compared to that of the neem and untreated check plot. In addition to moderately control the *P. xylostella*, Bt is safe for the natural enemies. After the training, almost all the participants understood more about insect pests of cabbage, and they were able to differentiate the pests and natural enemies. They are also willing to adopt bio-pesticides, if made available.

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SESSION 7

Genomic and other novel approaches to crucifer pest management

Baseline susceptibility of diamondback moth to the Cry1Ac protein and efficacy of *Bt* cauliflower

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ABSTRACT
Diamondback moth, *Plutella xylostella*, is a major destructive pest in India attacking cole crops such as cabbage and cauliflower. The larvae feed on leaves and cause extensive damage, skeletonizing the leaves when left unmanaged. The management of this pest is through insecticide sprays which lead to development of resistance to most insecticides. The alternative strategy is to deploy *Bt* crucifers which require an effective resistance management strategy to ensure longevity of the technology. Insect-resistant *Bt* cauliflower carrying a *cry1Ac* gene was developed through *Agrobacterium* – mediated co-transformation method and subjected to bioassays using the larvae of diamondback moth. The transgenic lines demonstrating 100% larval mortality and those that displayed normal Mendelian inheritance of *cry1Ac* gene were chosen for further analysis. The risk mitigation plan for insect-resistant transgenic crops requires baseline information on the target insect pests. The objective of this study was to estimate baseline susceptibility of diamondback moth for the Cry1Ac protein. The Cry1Ac susceptibility was determined from fifteen populations collected from cauliflower/cabbage growing regions in India, using leaf dip bioassay method. The bioassays were carried out with approximately 48-hr-old larvae of the field-collected populations, and mortality was evaluated after 72 hrs. There was 5.7-fold inter-population variation in the insect susceptibility to the Cry1Ac protein indicated by mortality. The estimated LC_{50} values were between 0.121-0.684 µg/ml of Cry1Ac protein. The baseline data was used to estimate the diagnostic concentration, LC_{99}, that causes 99% mortality.

Keywords
Diamondback moth, co-transformation, Cry1Ac, baseline susceptibility, *Bt* Cauliflower

INTRODUCTION
The diamondback moth, *Plutella xylostella* (L) is an economically important pest of cruciferous plants throughout the world. In India, cauliflower is grown on about 349000 ha and cabbage on about 310000 ha (Anonymous 2010).The young larvae feed on epidermal leaf tissue producing membranous patches. Later stage larvae feed on cabbage heads and cauliflower buds resulting in undersized cabbage heads and cauliflower. The damage caused by DBM results in an annual loss of $16 million in India (Mohan and Gujar 2003). Diamondback moth causes an average of 52% loss to marketable yields of cabbage (Kumar et al. 1983). In India yield losses in cabbage and cauliflower due to DBM can reach 90% if no chemical is used and 35% when used (Sandur 2004).

DBM management primarily relies on spray of insecticides. Most farmers (92 %) do not follow pest scouting before the sprays and tend to spray until the produce is harvested, irrespective of its necessity. The cost of pesticides accounts for one third of the total input cost and just under one-fourth of the total cost of cultivation (Sandur 2004).

DBM has the ability to develop high levels of resistance in a short time (Fahmy et al. 1991). Efforts to control this pest solely through conventional insecticides led to resistance development to most insecticides available in India (Singh 2002). DBM has been reported to develop resistance to various insecticides *viz.*, DDT (Verma and Sandhu 1968), cypermethrin, deltamethrin and fenvelarate (Balasubramani et al. 2008; Saxena et al. 1989; Vastrad et al. 2003; Raju and Singh 1995; Chawala and Joia 1991) quinalphos, monocrotophos, chlorpyriphos (Renuka and Regupathy 1996; Vastrad et al. 2003; Joia et al. 1994) and endosulfan (Kalra et al. 1997). Also, some of the management practices such as crop rotation, trap cropping with Indian mustard, use of light traps or pheromone traps, are either not preferred by the farmers or labor intensive. Management options being limited, one of the safe and effective alternatives is use of *Bt* cole crops. *Bt* cole crops have been developed and tested for more than 15 years (Metz et al. 1995) and have proven their potential for integrated pest

management (IPM) systems (Shelton et al. 2008). *Agrobacterium*-mediated co-transformation system for the generation of marker-free transgenic cauliflower plants has been validated at Maharashtra Hybrid Seeds Company Ltd (Mahyco). *Agrobacterium*-mediated genetic transformation of cauliflower was reported by Chakrabarty et al. (2002), Bhalla and Smith (1998) and Ding et al. (1998).

The objectives of this study were to establish baseline susceptibility of DBM populations to Cry1Ac protein present in *Bt* cauliflower, generation of marker-free insect-resistant transgenic cauliflower carrying *cry1Ac* gene using *Agrobacterium* -mediated co-transformation and test the efficacy of *Bt* cauliflower on DBM.

MATERIALS AND METHODS

Tissue culture and *Agrobacterium* - mediated co-transformation

A proprietary cauliflower line of Mahyco was used in transformations. The cauliflower transformations were performed using a modified protocol developed at Mahyco Research Centre based on protocols reported by Metz et al. (1995) and Bhalla and Smith (1998). The cotyledons from *in vitro*-grown four-day-old seedlings were inoculated with *Agrobacterium tumefaciens* strain LBA 4404 carrying *cry1Ac* gene in T-DNA of one plasmid and *nptII* and *GUS* genes in T-DNA of another plasmid. Both these plasmids were mobilised into the same *Agrobacterium* cell. The *cry1Ac* gene was driven by an enhanced 35S promoter.

The hardened plants were screened by Double Antibody Sandwich (DAS) ELISA for presence of Cry1Ac protein using the kit manufacturer's protocol (DesiGen Diagnostics, India) and GUS assays for expression of β-glucuronidase (Jefferson et al. 1986). Seedlings were raised from seeds (T_1) collected from primary transformants (T_0). The leaf discs from these seedlings were used for the GUS assays and ELISA.

Molecular analysis and identification of marker-free plants

To establish the marker-free status, T_2 seeds from marker-free lines were surface sterilized and inoculated at the rate of 10 seeds per bottle on MS0 medium (MS basal salts, B5 vitamins, 3% sucrose, 0.8% Agar, pH 5.7) in bottle supplemented with 50mg/L kanamycin (MS0K). The seedling growth was recorded 20 days after inoculation. The non-transgenic seeds were inoculated on MS0K medium as negative control. The seeds from all the tested plants were inoculated on MS basal medium (MS0) without kanamycin to ensure the viability of the seeds. The samples were also screened by PCR using *nptII* gene specific primers for reconfirming the marker-free status.

Tissue bioassays

The leaf discs from the transformed plants were used in the bioassays to test the efficacy of the inserted gene, *cry1Ac*, on DBM larvae. First instar larvae were used in the bioassays and there were three replicates per assay. The transgenic lines demonstrating 100% larval mortality and those that displayed normal Mendelian inheritance of *cry1Ac* gene were chosen for further analysis.

Insect collections for baseline susceptibility studies

Field populations of DBM were collected from cabbage and cauliflower crops from different regions of India (Table 1). Infested leaves with late instar larvae or pupae were collected from each location.

Table 1. Collection details of *P. xylostella*

Sr. No.	Location	State
1	Ahmednagar	Maharashtra
2	Bangalore	Karnataka
3	Belgaum	Karnataka
4	Coimbatore	Tamil Nadu
5	Delhi	Delhi
6	Haveri	Karnataka
7	Jaipur	Rajasthan
8	Jatheri	Uttar Pradesh
9	Kallakal	Andhra Pradesh
10	Nasik	Maharashtra
11	Pune	Maharashtra
12	Sabarkantha	Gujarat
13	Shankarpally	Andhra Pradesh
14	Varanasi	Uttar Pradesh
15	24 Paraganas	West Bengal

The collected populations were brought in to the laboratory and reared on cauliflower leaves at 26 ± 1^0C with 60% RH. The adults that emerged from pupae were released into breeding cages for oviposition. The neonates that emerged from eggs were allowed to grow to 24-48h-old larvae and used in leaf dip bioassays.

Leaf dip bioassays

Leaf dip bioassays were conducted to determine the response of *P. xylostella* larvae to Cry1Ac protein. Serial dilutions of Cry1Ac protein were made from primary stock (250 ppm). The source of Cry1Ac protein used in the bioassays was the commercial formulation, MVP II® (Mycogen Corp., USA), which contained 19.7% (by weight) Cry1Ac. The leaf discs were dipped in different concentrations of Cry1Ac protein for 20 s and allowed to air dry for one hour at room temperature. Treated leaf discs were placed in individual Petri-plates containing Whatman No.1 filter paper. Control leaf discs were dipped in distilled water containing 0.1 %

triton X-100. Six larvae were released on each leaf disc in a Petri-plate and allowed to feed for 72 hrs at 26 ± 1^0c, 60% RH. Larval mortality was recorded at 72 hours after release.

Statistical analysis

Mortality data were analyzed by probit analysis (Finney 1971) using POLO-PC (LeOra software 1987). Median lethal concentrations of mortality (LC) at 50% and 95% level were estimated for each population.

RESULTS AND DISCUSSION

Three primary transformants which were ELISA positive for Cry1Ac protein were regenerated. In T_1 generation, seedlings from all three primary transformants segregated in 3:1 (Cry1Ac positive: negative) as a single dominant gene in seedlings analysed (Table 2) and marker-free plants were identified in two lines.

Marker-free transgenic plants were found to be negative in GUS assays and PCR using *nptII* primers (data not shown). The growth inhibition of seedlings on medium with kanamycin was scored 20 days after seed inoculation. The seeds (T_2) from marker-free plants germinated and grew up to cotyledonary stage on medium containing kanamycin (Fig 1D) The marker-free plants and non-transgenic control plants did not grow further on kanamycin-containing medium (MS0K). As expected, the negative control plants (non-transgenic seeds on MS0K medium) also grew up to cotyledonary stage and further growth was arrested (Fig 1B). The seeds from non-transgenic control seeds and marker-free line grew beyond cotyledonary stage on medium without kanamycin (MS0) and this ensured the viability and quality of the seeds (Figs 1A and C).

We have generated transgenic cauliflower by *Agrobacterium*-mediated co-transformation and identified marker-free transgenic lines resistant to larvae of diamondback moth. The transgenic *cry1Ac* gene expressing leaves showed complete protection from the target pest in the bioassays (Fig 2). There was 100% mortality observed in the bioassays with transgenic cauliflower leaf discs.

Susceptibility of DBM populations exposed to various concentrations of Cry1Ac protein is presented in Table 3. The Cry1Ac susceptibility was measured in terms of LC (lethal concentration). The LC_{50} values ranged from 0.12 to 0.68µg/ml among the different populations. The highest LC_{50} was recorded at Haveri (0.68µg/ml) while the lowest was from Coimbatore (0.12µg/ml). The variation in the LC_{50} values among the field-collected populations was 5.7 fold and when compared to the LC_{50} value of lab population, variation was 17-fold. Phani Kumar and Gujar (2004) reported 29-fold variability in Cry1Ac susceptibility (LC_{50}) values among the populations with values between 0.011-0.324 µg/ml Cry1Ac protein. Leaf dip bioassays carried out by Perez et al. (1997) found that there was 828.5-fold variation among the populations. The variation in LC_{50} values in our study was lower as compared to the values reported by Phani Kumar and Gujar (2004) and Perez et al. (1997). The variability observed could be natural variability existing in the populations, or sampling variation. The bioassay data were used to estimate diagnostic dose (LC_{99}) for resistance monitoring and it was estimated to be 29.81 µg/ml.

CONCLUSIONS

The marker-free *Bt* cauliflower expressing a Cry1Ac protein developed by Mahyco was found to cause 100% mortality of DBM larvae. The baseline susceptibility data demonstrated that the variability found among the populations is low and the estimated diagnostic dose of 29.81µg/ml can be used in resistance monitoring programs in the future. The marker-free Cry1Ac cauliflower may prove useful in IPM for India. However, it should be noted that because of the history of resistance evolution to *Bt* proteins in DBM there is concern about the deployment of single *Bt gene* plants. Thus, transitioning to plants expressing pyramided *Bt genes*, especially those not previously used in *Bt* sprays, would enhance the durability of this strategy.

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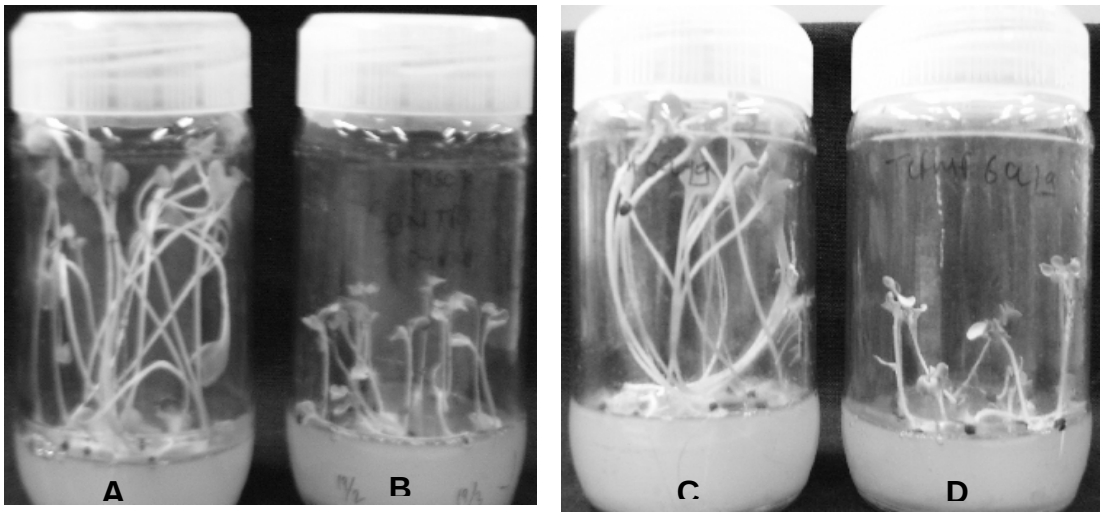

Figure 1. Kanamycin sensitivity assay *in vitro* based on cauliflower seed germination from control and marker free lines A. Non transgenic cauliflower seed germination on MS basal medium (MS0) without kanamycin, B. Non transgenic seed germination on MS basal medium with kanamycin (MSOK), C. Transgenic marker-free seed germination on MS0 and D. Transgenic marker-free seed germination on MS0K

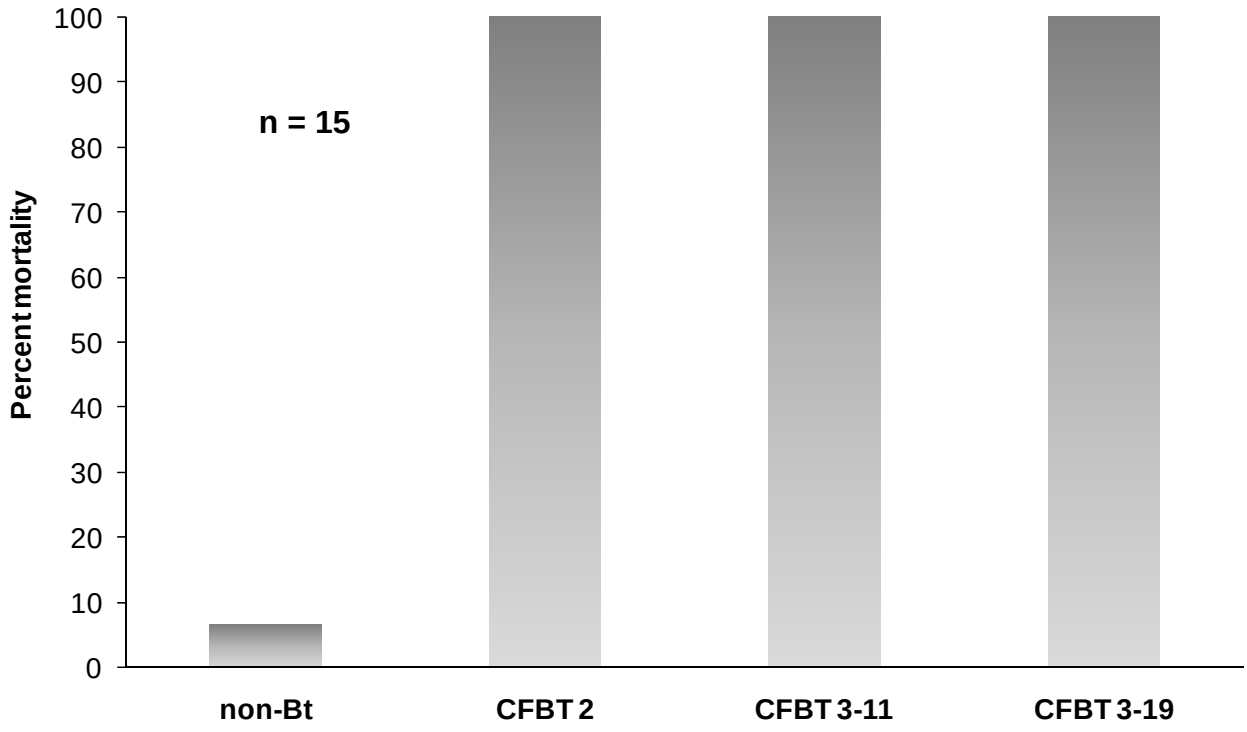

Figure 2. Percent mortality of larvae in bioassays with elite marker-free *Bt* cauliflower events

Table 2. Segregation analysis based on ELISA for Cry1Ac protein for T_1 generation seedlings

Plant /Event ID	Number of Seedlings Tested			χ^2 value (3:1)	P value
	Total	ELISA+	ELISA-		
CFBT 1	25	20	05	0.32 ^{ns}	0.57
CFBT 2	28	20	08	0.19 ^{ns}	0.66
CFBT 3	30	19	11	2.17 ^{ns}	0.14

ns indicates non-significance at P>0.05

Table 3. Susceptibility of *P. xylostella* larvae collected from different locations to Cry1Ac protein

Population	**N**	χ^{a}	**Slope (± SE)**	**LC_{50} (95%CI)[b]**	**LC_{95} (95% CI)[b]**
Bangalore	971	6.77*	1.29 ± 0.07	0.21 (0.16-0.28)	3.92 (2.62-6.54)
Belgaum	535	15.37*	1.17 ± 0.10	0.58 (0.31-1.06)	14.61 (5.67-85.29)
Coimbatore	630	2.68	1.31 ± 0.09	0.12 (0.10-0.16)	2.18 (1.49-3.53)
Delhi	584	10.11*	1.25 ± 0.10	0.51 (0.33-0.79)	10.56 (5.14-32.56)
Haveri	532	10.53*	1.34 ± 0.11	0.68 (0.44-1.07)	11.68 (5.70-37.60)
Jaipur	491	35.83*	1.32 ± 0.10	0.17 (0.09-0.30)	2.94 (1.03-29.85)
Jatheri	927	3.66	1.29 ± 0.07	0.31 (0.23-0.38)	5.85 (4.26-8.55)
Kallakal	536	4.01	0.94 ± 0.08	0.18 (0.13-0.25)	9.98 (5.39-22.93)
Ahmednagar	731	7.95*	0.86 ± 0.07	0.28 (0.18-0.45)	23.19 (9.38-90.99)
Nasik	545	7.09*	1.10 ± 0.10	0.46 (0.30-0.69)	14.42 (6.96-42.87)
Pune	688	5.87	1.08 ± 0.08	0.58 (0.44-0.76)	19.01(11.06-38.70)
Sabarkantha	550	14.54*	1.14 ± 0.10	0.34 (0.18-0.62)	9.67 (3.85-50.27)
Shankarpally	1033	22.97*	1.00 ± 0.06	0.27 (0.16-0.48)	12.14 (4.78-55.48)
Varanasi	970	26.50*	1.28 ± 0.07	0.14 (0.10-0.18)	2.66 (1.27-8.64)
24 Paraganas	1140	6.05*	1.20 ± 0.06	0.14 (0.11-0.17)	3.22 (2.19-5.18)
Lab	540	7.43*	1.28 ± 0.11	0.05 (0.03-0.07)	0.95 (0.53-2.22)

[a] Chi-square goodness-of-fit as determined using POLO-PC and departures from an expected model based on heterogeneity factor >1.0
[b] µg/ml Cry1Ac in diet with 95% confidence intervals (CIs) at the 50% levels of probit mortality.
* Chi-square is significant (P<0.05)

The efficacy and sustainability of the CIMBAA transgenic Cry1B/Cry1C Bt cabbage and cauliflower plants for control of lepidopteran pests

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ABSTRACT
In 2003 the Collaboration on Insect Management for Brassicas in Asia and Africa (CIMBAA) public/private partnership selected the Cry1B/Cry1C Bt protein combination as having the potential to provide effective and sustainable control of diamondback moth (DBM), *Plutella xylostella*. Following transformations and extensive plant selection, insect efficacy trials were undertaken in 2008 to 2010 in north India (Murthal near New Delhi) and south India (near Bengaluru) in large scale screen-house experiments using artificial infestations on the best performing (Elite Event) plant lines and on hybrids produced from them. Plant damage was scored on a scale of 0 (no visible damage) to 4 (plant effectively destroyed). For DBM, cabbage cluster caterpillar (*Crocidolomia binotalis*), cabbage webworm (*Hellula undalis*) and semi-looper (*Trichoplusia ni*) there was zero insect survival and a zero damage score on the Elite Event lines and on their hybrids, while control plants had 50 to 100% insect survival (depending on species, life stage and trials) and damage scores of 3.3 to 4. Cabbage white (*Pieris brassicae*) and cotton leaf worm (*Spodoptera litura*) showed some larval survival and damage scores up to 1.4 (especially in early trials) but no survival to pupation. Screening of DBM populations worldwide (inc. 18 populations for Cry1B and 13 for Cry1C from India) showed mean LC_{50}s close to that of international susceptible strains. To date F2 screening has not identified the presence of resistance genes in DBM in the field. Cry1B resistance was slowly developed artificially in the laboratory but 1C resistance and resistance to the Cry1B/1C combination was harder to develop and had higher fitness costs. The 'resistant' lines showed some extended survival of stunted DBM larvae on dual gene Bt plants but no survival to pupation. There was no cross-resistance between Cry1B and Cry1C. Resistance to both genes was autosomal and recessive. Beneficial insects were demonstrated to have the potential to provide additional mortality on rare surviving insects in Bt fields. Aphids were well controlled for the first 40 days post-transplanting using imidacloprid pelleted onto seed and, if necessary, by 1-2 *Verticillium lecanii* sprays thereafter. Surviving *S. litura* and *Helicoverpa armigera* in Bt sprayed fields were well controlled by one or two applications of their species-specific nucleopolyhedroviruses

Keywords
Cry1B/Cry1C, brassicas, diamondback moth, CIMBAA

INTRODUCTION
Grzywacz *et al*. (2010) summarize the current pest management practices for brassica production in Asia and Africa and review the rationale for the deployment of Bt cabbage and cauliflower. Studies emerging from the Shelton laboratory at Cornell University have shown excellent control of *Plutella xylostella* by *Brassica oleracea* plants carrying a synthetic or modified *Bt* gene (see references in Shelton *et al*. (2008). Transgenic collards with *cry1Ac* or *cry1C* genes showed complete control of susceptible *P. xylostella* larvae (Cao et al. 2005). Additional studies with a *cry1C* gene expressed in broccoli, demonstrated control of Cry1Ac-resistant *P. xylostella* (Cao *et a*l. 1999) and studies with pyramided *cry1Ac* and *cry1C* broccoli plants demonstrated excellent control of both Cry1C-resistant and Cry1Ac-resistant *P. xylostella* (Cao et al. 2002, Zhao *et al*. 2003). However, *P. xylostella* has shown its ability to develop resistance to sprayed Bts in the field (Mau and Gusukuma-Minuto 2004). In developing a Bt-based resistance to *P. xylostella*, it was therefore essential to ensure the sustainability as well as the immediate efficacy of the particular gene combination chosen. Russell *et al.* (2008 and this volume) provide an overview of the Collaboration on Insect Management for Brassicas in Asia and Africa (CIMBAA), the rationale for the choice of the Bt proteins Cry1B and Cry1C and the progress in other areas of the collaboration. Shelton *et al*. (2009) summarize the efficacy of the individual Cry1B and Cry1C purified proteins against the major caterpillar pests of brassicas in Asia, Africa and elsewhere. The

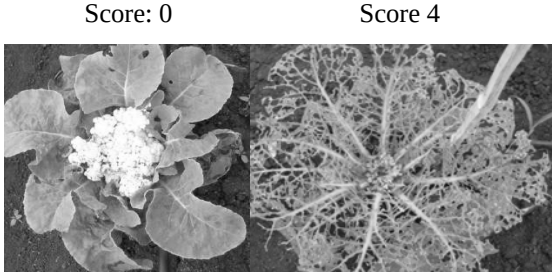
current paper provides detail on the efficacy of CIMBAA transgenic cabbage and cauliflower containing this Cry1B+Cry1C gene combination against these pests and explores its likely sustainability in the face of evolved resistance, with preliminary information on advances in the development of an appropriate IPM context for the control of pest insects not susceptible to these Bts.

EFFICACY OF Bt PLANTS

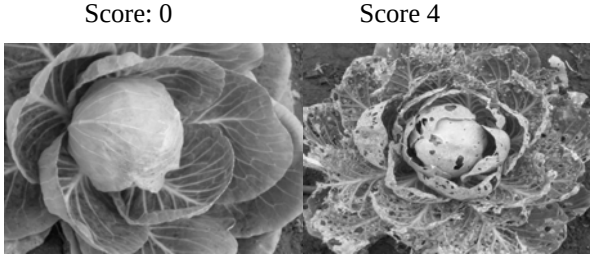
Methods

The various cabbage and cauliflower lines derived from different transformation events were selected for their suitability for commercialization (Elite Events) in large-scale screenhouse trials from 2008-2010 on the CIMBAA private partner (Nunhems India Pvt Ltd) sites at Murthal near New Delhi in the Rabi (Spring) season and near Bengaluru in the Kharif (Autumn-winter) season.

Colonies of the key pest species (P. *xylostella, Crodidolomia binotalis, Hellula undalis, Pieris brassicae* and *Trichoplusia ni*) were maintained at Nunhems facilities or species such as S*podoptera litura and Helicoverpa armigera* were obtained from the laboratory colonies of Pest Control India Ltd. The screen-houses were divided in half, each for cabbage and cauliflower and each half into plots for each pest species with three or more replicated sub plots of 15 plants per species tested in each trial for each line under selection.



a) Cauliflower



b) Cabbage

Figure 1. Damage scoring system from 0 (no damage) to 4 (totally useless for any commercial purpose) as measured 10 days after release of insects (in this case 40 neonates of *S. litura* per plant). a) cauliflower b) cabbage

Insects were released onto each plant using eggs on sheets (*P. xylostella* 50/plant; *H. undalis 15* or 20 per plant; *P. brassicae* 60/plant) or neonate larvae (*C. binotalis* 10 or 15/plant; *T. ni* 10 or 15/plant; *S. litura* 40/plant). Counts were made on surviving larvae on the 5th and 10th days following release (7th and 12th day for *P. xylostella*). Plant damage scores were taken at the second observation, on a scale of 0 (no feeding damage seen) to 4 (plant effectively destroyed for any commercial purpose (Figure 1).

Results

The results for the finally selected Elite Event and the hybrids produced using that line as one parent are presented in Table 1.

In summary, all non-Bt control lines were very heavily damaged by all species. There was no larval survival or damage at all on Bt cabbage or Bt cauliflower or their hybrids for *P. xylostella, C. binotalis* and *H. undalis,* the three most widespread and important caterpillar pests of brassicas, nor for *T. ni* which is a more sporadic problem. There was some survival of *P. brassicae* larvae in 2008 on Bt cabbage but none in 2010 and none in either year on the Bt cauliflower. None of the surviving larvae on Bt cabbage in 2008 survived to pupation. (Note: the 2008 cabbage may still have been segregating for Bt in these early trials). There was 3% survival of *S. litura* larvae on Bt cabbage and its hybrids in 2009 in the N. India trials in 2009 but damage scores were less than one, with even lower survival and damage on cauliflower. Again there was no survival through pupation on either Bt cabbage or Bt cauliflower. Larvae of any species which did survive on Bt plants were small and stunted.

These results suggest that the Cry1B/Cry1C combination is capable of providing excellent control of the major caterpillar pests of cabbage and cauliflower, particularly given that in the artificial screen-house situation there was very strongly reduced natural mortality from parasitoids or predators. (See below for details on IPM strategies for other pests).

SUSTAINABILITY OF Bt PLANTS

Risk of resistance development in diamondback moth

P. xylostella has a long history of development of resistance to insecticides including Bts (Mau and Gusukuma-Minuto 2004) and it has proved possible to select very high levels of resistance to Cry1C in the laboratory (Zhao et al. 2001). For us to have confidence that the Cry1B/Cry1C combination would stand up against the risk of resistance development in a field situation, the following assumptions required to be met (essentially those of the 'high dose-refugia strategy' as used in other commercialized GM crops).

- *Expression of insecticidal protein in all attacked parts of the plant is high relative to the susceptibility of the pest*

- *Genes for resistance to either protein are rare in the field pest population*
- *The stacked proteins are not cross-resisted*
- *Proteins make an equal contribution to mortality, and are approximately equally susceptible to resistance development*
- *Proteins do not act antagonistically*
- *Resistance is functionally recessive*
- *Beneficial insects will contribute to mortality of insects resistant to the Bt proteins*
- *Plant host diversity within the agro-ecosystems will make planted refugia unnecessary*

Expression of insecticidal protein in all attacked parts of the plant is high relative to the susceptibility of the pest

Susceptibility of the major caterpillar pests, including *P. xylostella* to Cry1B and Cry1C protein was reported in Shelton *et al*. (2009). The Energy and Resources Institute, New Delhi (Kaushik *et al*. In Prep) confirmed that the Bt proteins were expressed in all plant tissues (including curds in cauliflowers) and uniform as well as at high enough levels to ensure *P. xylostella* mortality in all tissues and at all plant stages. The screen-house trials described above showed these plants to be effective in caterpillar control, with concerns only for *S. litura* and particularly for *H. armigera*.

Genes for resistance to either protein are rare in the field pest population

Shelton *et al*. (2009) showed that despite the c.100 fold variation in LC_{50}s across *P. xylostella* field populations in USA, China, Australia, Indonesia, Taiwan and India, all LC_{50}s were < 1.0 ppm for Cry1B and <1.2ppm for Cry1C and so no more than seven times the LC_{50} of international standard susceptible strains (0.43 ppm for Cry1B and 0.18 ppm for Cry1C). Screening at the Indian Agricultural Research Institute using the F2 method of Andow and Alstad (1998) of Indian populations from the mountainous north (Katrain, Himachel Pradesh) and the plains (Hosur – Tappi-Manali) was not able to identify resistance genes, although this work is still on-going.

The stacked proteins are not cross-resisted

Using the *P. xylostella* strains selected in the laboratory which were moderately resistant to Cry1B and Cry1C, experiments in Australia and India have showed no cross resistance between Cry1B and Cry1C in either direction (Behere and Gujar in prep.).

Proteins make an equal contribution to mortality and are approximately equally susceptible to resistance development

The range of susceptibilities of different *P. xylostella* populations to the two Cry proteins individually is given in Shelton *et al*. (2008). The mean and range of LC_{50}s for Indian populations is given in Table 2.

Table 2. LC50s of Indian populations of *P. xylostella* and an international susceptible standard (Geneva) strain (data ex Shelton *et al*. 2008)

		Mean LC50 ug/ml pure Cry protein		
	No. Indian populations	'Geneva' strain	Mean of Indian populations	Range of means of Indian populations
Cry1B	18	0.43	0.09	0.01-0.46
Cry1C	13	0.18	0.18	0.01-0.61

For Indian strains, on an average, Cry1B showed 1.6 times the efficacy of Cry1C against *P. xylostella*. However, in the CIMBAA plant lines examined in detail for Bt expression, the proportion of the two proteins produced by the plant averages c. 0.66 Cry1B : 1.0 Cry1C in cabbage and 0.25 Cry1B : 1.0 Cry1C in cauliflower (Kaushik *et al*. in prep). As a happy consequence, their killing efficacy (the product of the expression x the LC_{50}) is therefore in the ratio of Cry1B: Cry1C 1.0:1.0 in cabbage and 0.4:1.0 in cauliflower – probably sufficiently close to contributing equal mortality for the purposes of risk of resistance selection.

Laboratory selections with *P. xylostella* using pure Cry1B and Cry1C proteins separately and together were undertaken at Melbourne University and in the Indian Agricultural Research Institute. Selection was continued though 21 generations for Cry1C and Cry1C+B and for 25 generations for Cry 1B. For Cry1C and Cry1B+C resistance was so hard to select that levels peaked at 8 fold resistance in the 10th generation of selection and declined rapidly thereafter as selection had to be relaxed to allow sufficient survival for breeding. Insects selected with Cry1B protein showed 54-fold resistance over the unselected Australian (Waite) strain of the same genetic background which 304 fold resistance with respect to a field-derived (Queensland) strain and 1,290 fold resistance in comparison with the most susceptible (Hosur) Indian strain.

Resistant insects selected in the laboratory in both Australia and India were very delicate and did not breed well. As soon as selection was relaxed, the resistance levels declined sharply (*i.e*., there was a strong fitness cost of resistance). The fitness cost with Cry1B and Cry1C resistance was in the form of extended larval growth, abnormal development of pupae and adults, and less fecundity in females. For Cry1C and Cry1B+C resistance was very hard to select *i.e*., fitness costs were close to 100%. Cry1B resistance fitness cost was around 60% in the laboratory but would most likely be much more in the field.

Proteins do not act antagonistically

Experiments in Australia and India with Cry1B and Cry1C pure protein used together in ratios around that expressed in the plants (Cry1B:Cry1C, 1:1, 1:2 and 2:1) showed only additive effects on *P. xylostella* mortality.

Resistance is functionally recessive

At the University of Melbourne the Cry1B resistant strain was crossed with the susceptible Australian (Waite and Queensland) strains and with the Indian (Hosur) susceptible strain and the offspring back-crossed to the Cry1B resistant strain. Inheritance of resistance proved to be recessive with dominance co-efficient (h) values of around 0.24-0.29 when tested in different genetic backgrounds. For the much less resistant Cry1C strain, work at IARI suggested an h value of 0.4 (incompletely recessive) and reciprocal crossing of moths resistant to either Cry1B or Cry1C showed resistances to both to be autosomal and functionally recessive. We can conclude that to be resistant to the Bt plants (even to the modest level selected) an insect would require to be homozygous for the resistance gene. This had already been established for Cry1C resistance in experiments in USA.

Survival of 'resistant' diamondback moth on Bt plants

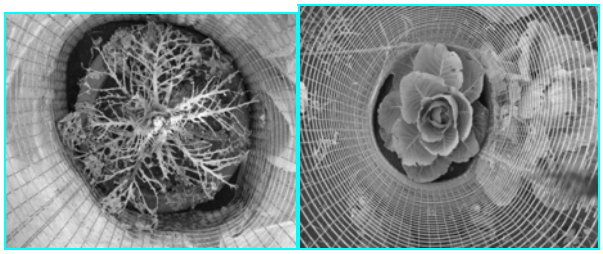
Challenging the three selected 'resistant' strains of *P. xylostella* with transgenic Bt plants in Australia and India produced no significant increased survival of the 'resistant' larvae of the Cry1B strain (>400 fold less susceptible than the Indian field strains) and the Cry1C strain (>20 fold less susceptible than Indian field strains), and only a lengthening of larval life by a few days in a few individuals of the Cry1B+Cry1C selected strain. No insects survived on the Bt plants through pupation to adulthood.

Beneficial insects will contribute to mortality of insects resistant to the Bt proteins

The potential role of natural enemies in removing initial, rare, surviving caterpillars in a transgenic cabbage or cauliflower field was unknown. Would beneficial insects be sufficiently common in Bt brassica fields to play a significant role? This was tested using the methods of Furlong *et al.* (2004 a&b).

The Energy and Resources Institute undertook trials in north (near Delhi) and south (near Bangalore) India, using simulated Bt cabbage and cauliflower fields (spraying Bt on non-transgenic crops) and examining the mortality of artificially placed 'rare' surviving *P. xylostella* in those fields. The presence of beneficial insects was measured in pitfall traps and by examining whole plants taken to the laboratory. Their impact on *P. xylostella* mortality was measured by the use of caged plants which were either partially open to access to predators and parasitoids or fully closed (Figure 2) and which had small numbers of laboratory reared *P. xylostella* released into them as eggs. Predators were collected and tested to ascertain whether they had eaten *P. xylostella* using a Polymerase Chain Reaction (PCR) diagnostic test provided by Dr Furlong from University of Queensland and validated at the University of Melbourne.

a)



b) **c)**

Figure 2. Parasitoid and predator exclusion cages infested with *S. litura* eggs (a) in place in a Bt sprayed field (b) a closed cage showing *S. litura* damage (c) a partially open cage with a lightly damaged plant due to the access to parasitoids and predators.

Major predators were spiders and ants. In the north India, predators were more numerous in simulated Bt fields than in the conventionally sprayed equivalents. Differences were smaller in South India. Mortality in open cages was 12% versus only 1% in the closed cages in the north India, but 39% in open cages in the south India versus 21% in the closed cages. In the north India, parasitoid induced mortality (especially by *Cotesia* sp.) was visible in the simulated Bt fields but not in the conventionally sprayed fields. In the south India, *Oomyzus* sp. was also important and again there was much more activity in the simulated Bt field than in the conventionally sprayed control. It is clear that these parasitoids and predators are having a considerable impact. However, unfortunately there was no background wild population of *P. xylostella* in the control fields in those seasons making interpretation of the results difficult and the trial is being repeated near Delhi in August - September 2011.

Plant host diversity within the agro-ecosystems will make planted refugia unnecessary

Most cabbage and cauliflower fields in the developing world are small, with farmers frequently planting a diversity of crops simultaneously and with cruciferous weeds present on verges, waste areas and sometimes within the crop. Mustard and radish are both *P. xylostella* hosts and are widely grown. Planted areas for each crop are available from the Indian Government Agricultural Statistics and 'ground truthing' searches for weed and crop alternate hosts were made by The Energy and Resources Institute in representative brassica production districts of North India (Sonipat district of Murthal in Haryana and at Hapur in the Ghaziabad district of Uttar Pradesh) and South India (Tehsil Malur in Kolar district of Karnataka). Planting times are naturally variable and mustard planting areas were difficult to ascertain but from radish plantings alone, when compared with the cabbage plus cauliflower areas, it would seem that a 'refuge' area of 45% would be available in Sonipat, 7% in Ghaziabad and 30% in Kolar. Mustard areas are expected to be comparable. Surprisingly, cruciferous weed hosts were not found to be significant in any area examined and no *P. xylostella* were found in systematic sweep netting off the crops.

The distance likely to be moved before mating by a rare, resistant *P. xylostella* individual emerging in a Bt cabbage field is unknown and these issues need further study, but unless market penetration of Bt cabbage and cauliflower in a particular district was very high indeed, it would seem that alternate crop hosts should have a valuable role to play in delaying resistance development.

Conclusion on Bt resistance risks for P. xylostella

Taken together these results suggest that a) development of simultaneous resistance to Cry1B and Cry1C in the field would be very difficult and b) resistant insects would be very 'unfit' in a natural environment c) both parents of a resistant insect would have to pass on a resistance gene, making the substantial non-Bt crop refugia important as a source of insects not carrying the resistance genes. These would be likely to mate with the rare resistant insects emerging from the Bt field and their offspring would be susceptible to the Bt plants, removing the resistance gene from the population gene pool. A version of visual basic resistance risk model (Kranthi and Kranthi 2004) *DBM-Bt-Adapt* has been produced for *P. xylostella* in India and resistance risk management scenarios are under virtual test now.

IPM strategies for Bt crops

Aphids are widely recognized as the second most important group of cabbage and cauliflower pests in the developing world (Grzywacz *et al.* 2010) including India (Badnes-Perez et al 2006) and they are not, of course, susceptible to Bt proteins. The key species for India are *Myzus persicae, Brevicoryne brassicae* and *Lipaphis erysimi.* Amongst the Lepidoptera, *S. litura* is not totally controlled before significant feeding has occurred on the Cry1B/Cry1C plants and *H. armigera,* which appears to be becoming more important as a pest of brassicas, is not at all well controlled. Some alternate strategies are required for these species if the secondary benefits of Bt brassicas in removing insecticidal chemistries which adversely affect beneficial organisms is to be retained. Preliminary studies were undertaken in 2009 and 2010 at the same sites and dates as the caterpillar control trials using Bt (Xentari®) sprays which contain Cry1C as a major component, to mimic transgenic Bt plants in the open field.

Aphids were well controlled for at least the first 45 days post transplanting by using imidacloprid (0.1%) to drench the soil in seedling trays prior to transplanting. However, this has operator health implications and, in any event, many farmers transplant bare rooted, which reduces the efficacy of the treatment. Imidocloprid (as Confidor® 600 FS) experimentally pelleted onto the seed, resolved both those issues resulting in a mean of only 3.7 aphids/plant at 40 days post transplanting as opposed to 90 aphids per plant in the control plots. In the second half of the season, where necessary, one or two sprays of *Verticillium lecanii* (at 45 and 55 days post transplanting) gave satisfactory control until harvest.

S. litura and *H. armigera* were artificially infested onto the sprayed Bt plots. Larvae surviving the Bt sprays were reduced by >90% due to applications of their species-specific NPVs at $1.04x10^9$ PIB. These NPVs are readily commercially available in India. It is likely that only spot spraying of hotspots of these caterpillars in Bt fields would be required, as caterpillars of both species are generally killed or severely stunted when feeding on Bt transgenic plants.

Using open and closed cages with artificial infestations of the pests, of the same type as used for *P. xylostella* (Figure 2) it was possible to separate the impacts of natural enemies, the Bt sprays and the additional mortality due to NPV. For *S. litura*,on both cabbage and cauliflower, mortality was around 30% at 10 days after infestation with neonates in the absence of any of the three mortality factors. Parasitoids and predators raised that mortality to around 70%. Bt sprays alone produced around 55% mortality and with predators and parasitoids this rose to c.90%. Bt + *S. litura* NPV in the absence of natural enemies produced 100% mortality. The addition of natural enemies could not of course increase this, but it did result in higher mortality in the days leading up to the 10 day post-treatment sample date.

Identical experiments with *H. armigera* produced similar results with c. 22% mortality in the absence of natural enemies, Bt or NPV rising to c80% with Bt + natural enemies and >97% mortality with all three factors operating. By 15 days post the start of the experiment this rose to 100% mortality on both cabbage and cauliflower.

The timing of attacks of these secondary pests varies with the geographic locations and timing of the crop and further work on locally adapted IPM packages will be required.

CONCLUSION

The transformation events producing the Cry1B/Cry1C cabbage and cauliflower lines eventually selected as suitable for use in generating commercial hybrids resulted in complete control (100% mortality) of early larval instar diamondback moth (*P. xylostella*), cabbage cluster caterpillar (C. *binotalis*), cabbage webworm (*H. undalis*) and cabbage looper (*T. ni*) caterpillars. Control of cabbage white butterfly (*P. brassicae*) and cabbage leafworm (*S. litura*) was good, with no survival through pupation in either species but resulted in some larval feeding damage.

Under laboratory conditions, resistance to Cry1C and to the Cry1B+Cry1C combination in *P. xylostella* was very difficult to select and unstable in the absence of selection and the resistant insects were difficult to maintain. Cry1B resistance was slightly easier to select but also unstable. There was no cross-resistance between the two proteins, which operated additively. Indian field populations of *P. xylostella* are highly susceptible to both Bt proteins and the F2 screening undertaken to date has not revealed the presence of resistance alleles. Laboratory selected *P. xylostella* several hundred fold more resistant to Cry1B, >20 times more resistant to Cry1C and several time more resistant to the Cry1B+Cry1C combination than Indian field strains, were unable to survive to pupation on the Bt plants. Alternate crop brassicas provide a very useful prospective 'refugia' .

Experimental seed coatings with imidacloprid offer good early season protection against aphids and *V. lecanii* applications are effective thereafter. The available NPVs of *S. litura* and *H. armigera,* in combination with Bt, provides excellent control.

In all, the Cry1B+Cry1C combination offers an excellent new tool in cabbage and cauliflower IPM.

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The authors would like to thank the dedicated field team at Nunhems stations in India for their painstaking work in insect testing and selection. Dr Gangadhar of Pest Control India Pvt. was most generous with advice and insect material. Dr M. Furlong of University of Queensland provided training and support in the predator/parasite field cage work. Dr V.Khalia and Ms R. Nair of IARI's Entomology Division undertook the Indian resistance assays. The work was partially supported by the Australia-India Strategic Research Fund grant BF020053 and by the UK Department for International Development.

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Table 1. Mean percentage larval survival (MLS) and plant damage score (DS) (*scale 0-4*) of pest species on Elite Event a) cabbage and b) cauliflower and their hybrids in trials near New Delhi (Kharif-Spring) and Bengaluru (Rabi- autumn) 2008-10 *(see text for details.)*

	CABBAGE							
	North India				South India			
	2008		2009		2009		2010	
	MSL	DS	MSL	DS	MSL	DS	MSL	DS
Plutella xylostella **Control**	76.8	3.6	72.8	4.0				
Elite Event	0.0	0.0	0.0	0.0				
Hybrid	-	-	0.0	0.0				
Crocidolomia binotalis **Control**			68.7	4.0	76.0	4.0		
Elite Event			0.0	0.0	0.0	0.0		
Hybrid			0.0	0.0	0.0	0.0		
Hellula undalis **Control**			4.0	4.0	4.0	4.0		
Elite Event			0.0	0.0	0.0	0.0		
Hybrid			0.0	0.0	0.0	0.0		
Trichoplusia ni **Control**			61.5	4.0	70.0	4.0		
Elite Event			0.0	0.0	0.0	0.0		
Hybrid			0.0	0.0	0.0	0.0		
Spodoptera litura **Control**	58.3	3.4	78.0	3.8	57.4	3.6		
Elite Event	1.4	0.2	3.2	0.6	8.6	0.5		
Hybrid	-	-	3.2	0.6				
Pieris brassicae **Control**	83.3	3.8					47.5	4.0
Elite Event	12.3	0.8					0.0	0.2
Hybrid	-	-					1.7	0.7

	CAULIFLOWER							
	North India				South India			
	2008		2009		2009		2010	
	MSL	DS	MSL	DS	MSL	DS	MSL	DS
Plutella xylostella **Control**	76.8	3.3	70.5	4.0				
Elite Event	0.0	0.0	0.0	0.0				
Hybrid	0.0	0.0	0.0	0.0				
Crocidolomia binotalis **Control**			67.0	4.0	84.0	4.0		
Elite Event			0.0	0.0	0.0	0.0		
Hybrid			0.0	0.0	0.0	0.0		
Hellula undalis **Control**			-	4.0	-	4.0		
Elite Event			-	0.0	-	0.0		
Hybrid			-	0.0	-	0.0		
Trichoplusia ni **Control**			83.5	4.0	68.0	4.0		
Elite Event			0.0	0.0	0.0	0.0		
Hybrid			0.0	0.0	0.0	0.0		
Spodoptera litura **Control**	89.6	3.64	71.9	4.0	67.5	4.0		
Elite Event	2.4	1.3	1.3	0.3	6.3	1.3		
Hybrid			1.4	0.4	18.5	2.1		
Pieris brassicae **Control**							51.3	4.0
Elite Event							-	-
Hybrid							1.3	0.1

Enhancement of the sterile insect technique using germ-line transformation technology

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ABSTRACT
The sterile insect technique (SIT) has been used for many years, with great success, against a number of pest insects. Among Lepidoptera, pink bollworm (*Pectinophora gossypiella*) and codling moth (*Cydia pomonella*) are current targets of SIT. There are, however, disadvantages associated with sterilisation by irradiation: it can have a negative impact on the field performance of released insects, both through chromosomal damage and extra handling; and radiation sources are expensive to run and keep secure. Using germ-line transformation technology, a variant of SIT called Release of Insects carrying a Dominant Lethal (RIDL) offers the prospect of the effect of sterilisation without irradiation. Using the tetracycline-repressible transcription control system, the dominant lethal gene can be repressed using dietary tetracycline (or suitable analogues) to allow a RIDL insect strain to be mass-reared efficiently. More importantly, male-only release can greatly improve the effectiveness of SIT in some species, presumably because released males will more eagerly seek a wild mate in the absence of sterile females. Classical genetics has provided SIT with the ability to release males only (in fruit flies, primarily): strains in which sex separation can be carried out prior to release. These strains have benefited SIT hugely for a small number of pest species, but the special chromosomes cannot be transferred to new species and must be made again from scratch. Furthermore, such strains typically suffer from problems with stability and productivity in mass-rearing. A variant of RIDL offers a similar sex separation tool without these disadvantages: such strains have been shown to be stable and productive, and the technology can be readily transferred to new species. Here, we outline prospects for development of this technology in the diamondback moth (*Plutella xylostella*).

Keywords
Sterile insect technique, transgenic, diamondback moth, *Plutella xylostella*

INTRODUCTION
The diamondback moth (DBM), *Plutella xylostella*, is the most significant global pest of cruciferous crops. The damage is caused by the larvae, which feed on the plant foliage until pupation.

Pesticides are the primary means of control, but DBM's capacity to rapidly develop resistance is notorious (Ankersmit, 1953; Johnson, 1953), and it has developed resistance to all known classes of insecticide (Shelton, 2001). This is largely due to the moth's short generation time, with up to 30 generations per year possible in ideal conditions (Ho, 1965). Furthermore, in many parts of DBM's range, host plants are treated with indiscriminate and unregulated pesticide use (Georghiou and Langunes-Tejeda, 1991; Mota-Sanchez et al., 2002), exacerbating the resistance problem.

With increasing chemical resistance and consumer demand for low chemical residues on vegetables, growers have an increasing need for chemical-free control methods against DBM. Deployment of natural predators, such as *Diadegma* and *Diadromus* species, has shown effectiveness against DBM. On the Atlantic island of St Helena, for example, release of *Cotesia plutellae* and *Diadromus collaris* led to dramatic decline of the local DBM population and consequent reduced use of chemical insecticides (Kfir and Thomas, 2001). Other chemical-free means of control are also employed. The fungus *Beauveria bassiana*, and baculoviruses are other biocontrol tools. Heavy watering can also help by drowning the feeding larvae (Talekar et al., 1986).

The sterile insect technique (SIT) offers another possible chemical-free option. SIT relies on the mass-rearing, sterilisation and release of large numbers of moths. This results in a reduction in the number of progeny in the subsequent generation and, if releases are conducted over a longer period of time, a collapse in the wild population. Previous studies have examined the sterilisation of DBM by gamma irradiation (Sutrisno and Hoedaya, 1993; Sutrisno et al., 1991) and the efficacy of SIT control in cages and the field using such moths (Nguyen Thi and Nguyen Thanh, 2001; Okine et al., 1998; Omar, 1991; Sutrisno, 2005; Yang et al., 2002). These experiments showed some promise for SIT, with Nguyen & Nguyen finding that releasing sterile males only is more effective that bi-sex releases. This is presumably because, for the

former, sterile males are not distracted from their intended task of finding and mating with wild females. As the authors comment, there is currently no means of producing large numbers of male-only DBM moths. Irradiation can also have a negative effect on insect performance (Cayol et al., 1999; Holbrook and Fujimoto, 1970; Hooper and Katiyar, 1971; Lux et al., 2002; Mayer et al., 1998), which would reduce the efficiency of SIT. Furthermore, due to a reliance on costly gamma radiation sources to induce sterility, SIT programmes require significant investment and are generally restricted to large-scale, area-wide control operations.

Here, we describe novel technology that could help overcome these issues for an SIT-type control method against DBM, and we report on our progress towards developing such technology in this pest moth.

Release of insects carrying a dominant lethal

A novel control method called Release of Insects Carrying a Dominant Lethal (RIDL®) (Thomas et al., 2000) works by the same principle as SIT, but avoids the need for the damaging irradiation step. Germ-line transformation technology, mediated by genetic elements called transposons, allows engineering of insect phenotypes, such as larval lethality. RIDL, initially developed in *Drosophila melanogaster* (Thomas et al., 2000) but now transferred to important pest species (Fu et al., 2007; Gong et al., 2005; Koukidou et al., 2008; Phuc et al., 2007), uses the tetracycline-repressible genetic system (Gossen et al., 1994; Gossen and Bujard, 1992, 2002) to repress lethal phenotypes. Supplying tetracycline or suitable analogues to the insect feed suppresses lethality and therefore permits rearing in a laboratory or mass-rearing facility. Withdrawing the dietary additive leads to induction of the lethal phenotype. In the case of a 'bi-sex' lethal RIDL strain, for example, the insects can be reared happily in the facility with tetracycline. After release of males and females into the wild, however, tetracycline is no longer available to their progeny and they die. Death of progeny has the same effect, in population terms, as parental sterility. This type of RIDL might, therefore, be considered a genetic equivalent of radiation-induced sterility, but without the costs to insect quality.

An SIT-type method also requires that released insects are marked, in order to distinguish them from wild insects in monitoring traps and thereby track their relative levels in the field. A key feature of RIDL strains is the inclusion of a visual and genetic marker, such as an expressed fluorescent protein.

RIDL strains also provide in-built biological containment. Rearing a pest insect in large numbers carries the risk of accidental release into the surrounding area. As RIDL strains are reproductively non-viable outside artificial rearing (in the absence of tetracycline), this risk is greatly reduced.

RIDL genetic sexing

For SIT, male-only sterile insect releases are generally considered to be more efficient than bi-sex releases. This is presumably because, for the latter, sterilised males may mate with their sterile female counterparts in transit to the field or after release, therefore distracting them from their intended task of mating with wild females. In addition, the females of many pest insects cause the pest damage: for example fruit flies damaging fruit when ovipositing and female mosquitoes transmitting disease when blood-feeding (males do not blood-feed). In the Mediterranean fruit fly (Medfly, *Ceratitis capitata*), female-lethal genetic sexing strains have been generated by chromosomal translocation (Franz, 2005). In the most commonly used strains, male-only collections of pupae can be produced by heat-treating them as eggs. This technology is, however, labour-intensive to develop and not readily transferrable to other species. The strains are also genetically unstable and tend to revert to wild-type over time.

RIDL strains that confer female-specific lethality have been developed in the Mediterranean fruit fly (Fu et al., 2007), the Mexican fruit fly (*Anastrepha ludens*) (Koukidou et al., 2008) and strains of the dengue-transmitting mosquito, *Aedes aegypti*, have been developed that show tetracycline-repressible female flightlessness (Fu et al., 2010). Transformation technology has facilitated development of these transgenic sexing strains, and similar phenotypes should be transferable into other species, including moth pests.

Working towards RIDL in diamondback moth

The first step towards development of RIDL in a new species is to generate transgenic strains by microinjection of DNA plasmid into pre-blastoderm embryos. Transformation events are mediated by use of transposable elements - in our case, the *piggyBac* transposon - co-injected with a source of transposase. These DNA constructs are non-autonomous and transposase is only made available at the initial transformation stage. Transgenic individuals are detected in the subsequent generation by screening for a transformation marker (e.g. a fluorescent protein). Other lepidoptera that have been previously transformed include the silk moth (*Bombyx mori*) (Tamura et al., 2000b), the pink bollworm (*Pectiniphora gossypiella*) (Peloquin et al., 2000) and the squinting bush brown butterfly (*Bicyclus anynana*) (Marcus et al., 2004).

This work has, thus far, produced transgenic insects at estimated efficiencies of 0.48-0.68%. These efficiencies – the number of independent lines expressed as a proportion of total G_0 survivors – is broadly comparable to that first achieved with *piggyBac* in other moths: pink bollworm (3.5%), *Pectinophora gossypiella* (Peloquin et al., 2000), and the silkworm (0.7-3.9%), *Bombyx mori* (Tamura et al., 2000a). With greater experience and

optimisation of transformation protocols, we might expect these rates to increase.

We used two fluorescent proteins – DsRed2 (red fluorescence) and ZsGreen (green fluorescence) (Clontech Laboratories Inc, USA) – as markers. To drive expression of these marker proteins, three promoter sequences were used: *Opie2*, a promoter fragment from the *ie2* gene of baculovirus *Orgyia pseudotsugata* nuclear polyhydrosis virus, a pathogen of the Douglas-fir tussock moth (*O. pseudotsugata*); Hr5*ie1*, a fragment of the *immediate-early*-1 (*ie*1) gene with the Hr5 enhancer, from the *Autographica californica* nuclear polyhydrosis virus (AcMNPV); and 3×P3, an artificial eye-specific promoter constructed using three tandem repeats of the binding site (P3) of the photoreceptor-specific Pax-6/Eyeless transcriptional activator (Sheng et al., 1997).

These marker cassettes worked well for the purposes of screening for transformation markers. Both proteins showed strong, easily screened fluorescence in all insect life stages, except early embryos.

Building on the transformation methods described here, we hope to generate DBM genetic sexing strains, with engineered properties similar to those described by Fu et al. in Medfly (2007).

RIDL in the field: precedents

The cotton pest moth, pink bollworm, has been controlled by SIT in southwestern USA for a number of years. We developed an engineered marker-only strain, called OX1138B, to provide a more reliable method of marking irradiated released moths.

To compare performance of OX1138B with the wild-type strain, called APHIS, currently used in the SIT programme, we co-released 1.1 million irradiation-sterilised moths of each strain over three cotton fields in Arizona over the course of a 2-month period (Simmons et al., 2011). As measured by recapture on pheromone traps, the transgenic strain's field performance – total recapture, persistence in the fields and dispersal ability – was comparable to that of APHIS. This trial showed that transgenic insect strains could perform well in the field. In total, OX1138B underwent open field trials for 4 years, during which over 2 million moths were released. The strain is currently under consideration for inclusion for use in the 2012 pink bollworm eradication programme.

In a 2010 experiment with the mosquito *Ae. aegypti* in the Cayman Islands, males of a bi-sex RIDL strain – OX513 - were released in a small town on Grand Cayman (Alphey, 2010). Monitoring of the wild population, by trapping adults and setting oviposition traps (to count eggs laid by wild females), showed that these RIDL releases were able to suppress the wild population by approximately 80% compared to non-treatment areas during the trial period. This proof of RIDL's efficacy provides encouragement for its prospects of controlling agricultural pests, such as DBM. Open-field releases of OX513 have, in 2011, also been undertaken in Brazil and Malaysia.

SUMMARY

We have achieved the first germ-line transformation of DBM, and now hope to use this technology to develop RIDL for control of this pest. In light of the pressures on growers to produce vegetables with little sign of pest acitivity and low chemical residues, such a control method could prove valuable in the future, as part of an IPM approach. In addition, development of transgenesis in DBM may open the door to use of this moth as a model species for wider scientific research.

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