Diamondback Moth and Other Crucifer Pests

Proceedings of the Second International Workshop, Tainan, Taiwan, 10-14 December 1990

Asian Vegetable Research and Development Center

The book...

In the Foreword of this book, the economic importance of diamondback moth is dramatically underscored with the estimate indicating its control probably costs US$1 billion annually. DBM larvae attack and severely damage the 42.2million tons of cabbage, cauliflower and broccoli grown worldwide. This book contains a unique collection of research papers aimed at better understanding the insect and thereby controlling its spread and damage to cruciferous crops.

The editor...

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N.S. Talekar, Editor

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Foreword

The global importance of diamondback moth is reflected in estimates that its control could cost approximately US$1 b annually. This insect attacks crucifers, particularly cabbages, broccoli, and cauliflower. The world production of these crops is over 42.2 million tons.

The resistance of diamondback moth to chemical sprays, and the growing concern about risks to farmers, consumers and the environment prompted AVRDC to initiate this important international meeting.

The conference, which attracted 200 scientists from about 30 countries, concentrated on advances in research to control this insect pest through integrated pest management techniques that rely less on chemicals and more on cultural improvements, host-plant resistance and biological control. This report constitutes a unique collection of material on worldwide efforts to control important insect pests of cruciferous crops.

AVRDC is pleased to have played a role in organizing this conference, and I want to thank our joint sponsors, the Council of Agriculture of the Republic of China, and the Food and Fertilizer Technology Center for the Asian and Pacific Region, and all those listed in the Acknowledgments, for their vital support.

Emil Q. Javier
Director General, AVRDC

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ECOLOGY AND HOST-PLANT INTERACTION

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Effects of Natural Enemies, Rainfall, Temperature and Host Plants on Survival and Reproduction of the Diamondback Moth

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Abstract

Life tables of the diamondback moth *Plutella xylostella* (L.) were developed in June, September and October 1989 in a broccoli field in Okayama, Japan. Four plots were set up to evaluate the role of natural enemies and the effect of precipitation on survival. One-third to one-half of the eggs and young larvae disappeared in the control plot from unknown causes. The mortality rate was lower in the plots without rainfall. The wash-off of eggs and larvae due to the direct impact of rain, and the drowning of young larvae after rains, were considered to be the major causes of mortality. A water sprinkling also washed off eggs and larvae on broccoli leaves. The percentage of wash-off of egg was higher on the leaves with a thicker layer of stearic acid. The percentage parsitism by *Cotesia plutellae*, *Diadromus subtilicornis* and *Tetrastichus sokolowskii* was high in the summer. Temperatures higher than 30°C delayed the development and reduced the survival of immature stages and fecundity of females. Diamondback moth fed on a wild crucifer *Capsella bursa-pastoris* had lower reproductive ability than those fed on cultivated crucifers.

Introduction

Life tables of the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), have been reported by several authors (Harcourt 1963; Iga 1985; Sivapragasam et al., 1988). They reported that most DBM larvae were killed in immature stages. However, the main mortality factor was different in each of the studies. Iga (1985) reported that seasonal fluctuation depended mainly on the action of natural enemies. However, Harcourt (1963), Nakagome and Kato (1975), Koshihara et al. (1982), and Sivapragasam et al. (1988) reported that precipitation severely affected survival of young stages. The wax bloom on leaf surface of host plants is removed by precipitation (Kimura 1987), and eggs laid on leaves may be washed off easily together with the wax bloom. Removal of wax bloom on the leaf surface promoted the oviposition by DBM (Eckenbrode et al. 1986). The marked decline in DBM population occurs annually in the summer season in Japan because of high temperatures (Yamada and Umeya 1972; Nakagome and Kato 1975).

In this study, we developed life tables of DBM by using the biological check method to evaluate the role of natural enemies in the field. In addition, in order to evaluate the effects of precipitation, temperature and nutrition of host plants on the survival of immature stages and reproduction of adults, experiments were also conducted under indoor conditions (Wakisaka et al. 1991).

Life table of DBM

Broccoli (*Brassica oleracea* var *italica*) was planted in a field (10 × 12 m) at Okayama University in western Japan (April-November 1989). Four plots, each 2 × 5 m, were set up and 4-week-old plants were transplanted at intervals of 1.0 × 0.5 m on 25 April, 6 August, and 4 September. No insect control measure was used except for the exclusion of natural enemies as mentioned below. Other cultural practices were those practised by local farmers. The kinds of plots were as follows: (l) Six plants in the first plot were caged individually by fine-meshed nylon gauze (30 cm in diam and 50 cm in height) to exclude all natural enemies and the entire plot was covered by plastic film 1 m above the host plants to prevent the effects of precipitation (Plot C). (2) The second plot was covered by plastic film 2 m above the plants to prevent the effects of precipitation (Plot R). Twenty plants were planted. (3) The third plot was enclosed by a plastic plate, 30 cm high, which was coated with tangle-foot to prevent the invasion of ground fauna by natural enemies. Twenty plants were planted (Plot G). (4) The fourth plot was maintained as an untreated check. Sixty plants were planted (Plot A).

In order to exclude natural enemies in plots C and G, methomyl was sprayed 1 week before the start of the experiment. At the start of the experiment, each plant was caged by nylon gauze after removing all insects from the plant, and two pairs of adults which had just emerged were released in the cage. Females were allowed to lay eggs for 3 days and the nylon gauze and adults were removed. The plants were observed every 2 or 3 days in June and September, and every 3 or 4 days in October. The eggs observed were marked individually on the leaf surface to check their fate. The number and life stages of individuals were recorded, as well as dead or parasitized ones. The larval stage of DBM was divided into 1st to 2nd instar and 3rd to 4th instars. When eggs and pupae did not develop within 1 week, they were collected and examined in the laboratory. Larvae and pupae which showed symptoms of granulosis virus (Asayama and Ozaki 1966) were regarded as diseased, and the individuals that had disappeared were attributed to unknown causes.

During the study period, the precipitation at egg stage was 51.5, 77.0 and 16.5 mm, and at early instars of larvae was 5.0, 7.0, and 10.5 mm in June, September, and October, respectively. The mean temperature was 25.3, 23.0 and 13. 9°C in the respective season.

The life tables in the early summer, summer and autumn are given in Tables 1-3.

Egg

The major mortality factor was from unknown causes in all seasons. The percentage parasitism by *Trichogramma chilonis* was highest in June, followed by September and October. Parasitism was lowest in plot R despite the fact that natural enemies could invade the plot. Predation by Chrysopidae and mites was observed, however the frequency was low. The rate of physiological death, i.e. unhatched, was as high as 15.8% in plot C in the summer.

First to second instar larvae

The mortality rate from unknown causes was highest irrespective of plots and seasons. The rate in plot C in June was as high as 29.9%. Predation of young larvae by four species of spiders, *Gnathonarium exsiccatum* Linyphiidae, Theridiidae and Tetragnathidae was observed, but it was rare. A few of the drowning young larvae were observed.

Third to fourth instar larvae

The percentage parasitism by *Cotesia plutellae* was as high as 50% in October. The old larvae were also attacked by *Dolichusha lensis*. Mortality from disease was low. The major mortality factor in June and October was attributed to unknown causes, although parasitism was highest in September. When we placed the sticky trap (10 × 10 cm) under the plants outside

Table 1. Life tables of DBM in the early summer (Rainy season, 21 May to 28 June) in 1989 (Wakisaka et al., 1991).

x	dxF	Plot C (6)[a]			Plot R (12)			Plot G (16)			Plot A (23)		
		lx	dx	100qx	lx	dx	100qx	lx	dx	100qx	lx	dx	100qx
Egg		105			420			266			818		
	Parasitism		0	0.0		26	6.2		49	18.4		168	20.5
	Predation		0	0.0		5	1.2		8	3.0		22	2.7
	Physiological death		3	2.9		4	1.0		4	1.5		18	2.2
	Unknown[b]		35	33.3		104	24.8		132	49.6		386	47.2
	Total		38	36.2		139	33.2		193	72.5		594	72.6
L_{1-2}		67			281			73			224		
	Predation		0	0.0		0	0.0		0	0.0		1	0.5
	Disease		0	0.0		0	0.0		0	0.0		0	0.0
	Unknown[b]		20	29.9		168	59.8		16	21.9		86	38.4
	Total		20	29.9		168	59.8		16	21.9		87	38.9
L_{3-4}		47			113			57			137		
	Parasitism		0	0.0		46	40.7		9	15.8		30	21.9
	Predation		0	0.0		0	0.0		0	0.0		1	0.7
	Disease		1	2.1		0	0.0		0	0.0		2	1.5
	Unknown[b]		3	6.4		21	15.6		23	40.4		63	46.0
	Total		4	8.5		67	56.3		32	56.2		96	70.1
Pupa		43			46			25			41		
	Parasitism		0	0.0		9	19.6		12	48.0		14	34.1
	Predation		0	0.0		22	47.8		5	20.0		18	43.9
	Disease		3	7.0		2	4.4		0	0.0		2	4.9
	Unknown[b]		0	0.0		0	0.0		0	0		0	0.0
	Total		3	7.0		33	71.8		17	68.0		34	82.9
Adult		40(38.1%)[c]			13(3.1%)			8(3.0%)			7(0.9%)		

[a]The number of plants. [b]Disappearance from unknown causes. [c]The survival rate from egg to emergence of adult.

Table 2. Life tables of DBM in the summer (4-28 September) 1989 (Wakisaka et al., 1991).

x	dxF	Plot C (4)[a]			Plot R (18)			Plot G (17)			Plot A (31)		
		lx	dx	100qx	lx	dx	100qx	lx	dx	100qx	lx	dx	100qx
Egg		171			473			274			962		
	Parasitism		0	0.0		17	3.6		2	0.7		150	15.6
	Predation		0	0.0		15	3.2		8	2.9		52	5.4
	Physiological death		27	15.8		20	4.2		14	5.1		50	5.2
	Unknown[b]		10	5.9		65	13.7		92	33.6		234	24.3
	Total		50.5	37	21.7		117	24.7		116	42.3		486
L_{1-2}		134			356			158			476		
	Predation		0	0.0		1	0.3		0	0.0		4	0.8
	Disease		2	1.5		0	0.0		1	0.6		2	0.4
	Unknown[b]		10	7.5		122	34.3		61	38.6		167	35.1
	Total		36.3	12	9.0		123	34.6		62	39.2		173
L3-4		122			233			96			303		
	Parasitism		0	0.0		106	45.5		47	49.0		156	51.5
	Predation		0	0.0		1	0.4		0	0.0		1	0.3
	Disease		0	0.0		0	0.0		0	0.0		0	0.0
	Unknown[b]		7	5.7		60	25.8		28	29.2		114	37.6
Total			7	5.7		167	71.7		32	78.2		271	89.4
Pupa		115			66			21			32		
	Parasitism		0	0.0		49	74.2		20	95.2		28	87.5
	Predation		0	0.0		13	19.7		1	4.8		3	9.4
	Disease		6	5.2		2	3.0		0	0.0		0	0.0
	Unknown[b]		0	0.0		0	0.0		0	0.0		0	0.0
	Total		96.9	6	5.2		64	97.0		21	100.0		31
Adult		109(63.7%)[c]			2(0.4%)			0(0.0%)			1(0.1%)		

[a]The number of plants. [b]Disappearance from unknown causes. [c]The survival rate from egg to emergence of adult.

Table 3. Life tables of DBM in autumn (4 October to 30 November) 1989 (Wakisaka et al., 1991).

x	dxF	Plot C (5)[a]			Plot R (10)			Plot G (10)			Plot A (15)		
		lx	dx	100qx	lx	dx	100qx	lx	dx	100qx	lx	dx	100qx
Egg		412			197			422			501		
	Parasitism		0	0.0		8	4.1		0	0.0		26	5.2
	Predation		0	0.0		13	6.6		14	3.3		24	4.8
	Physiological death		9	2.2		0	0.0		24	5.7		21	4.2
	Unknown[b]		78	18.9		43	21.8		150	35.6		177	35.3
	Total		87	21.1		64	32.5		188	44.6		248	49.5
L_{1-2}		325			133			234			253		
	Predation		0	0.0		0	0.0		0	0.0		0	0.0
	Disease		0	0.0		0	0.0		0	0.0		2	0.8
	Unknown[b]		34	10.5		15	11.3		90	38.6		83	32.8
	Total		34	10.5		15	11.3		90	38.6		85	33.6
L_{3-4}		291			118			144			168		
	Parasitism		0	0.0		2	1.7		5	3.5		4	2.4
	Predation		0	0.0		2	1.7		0	0.0		0	0.0
	Disease		0	0.0		1	0.9		0	0.0		0	0.0
	Unknown[b]		19	6.5		88	74.6		110	76.4		121	72.0
	Total		19	6.5		93	78.9		115	79.9		125	74.4
Pupa		272			25			29			43		
	Parasitism		0	0.0		4	16.0		10	34.5		9	20.9
	Predation		0	0.0		0	0.0		2	6.9		2	4.7
	Disease		89	32.7		0	0.0		3	10.3		3	7.0
	Unknown[b]		0	0.0		0	0.0		0	0.0		0	0.0
	Total		89	32.7		4	16.0		15	51.7		14	32.6
Adult		183 (44.4%)[c]			21 (10.7%)			14 (3.3%)			29 (5.8%)		

[a]The number of plants. [b]Disappearance from unknown causes. [c]The survival rate from egg to emergence of adult.

of the plot, six pupae were found on the trap. This suggested that some old larvae dispersed from host plants before pupation. Therefore, the unknown mortality may include dispersal of mature larvae.

Pupa

Parasitism by *Tetrastichus sokolowskii* and *Diadromus subtilicornis* was observed. Parasitism was highest, 74-95% in June, and lowest, 16-35%, in October. A ground beetle *Amara obscuripes* ate pupae. Most of the unknown disappearance of pupae may be due to bird predation (Iga 1985) because pigeons and sparrows often visited this field. Many pupae were infected with disease in plot C in the autumn.

Total mortality rate

The total mortality rate from egg to emergence of adults was higher in September than in June or October (plot A).

Effects of water sprinkling on the survival of DBM

In order to investigate the preference of egg-laying for the parts of host plants by female adults, 20 pairs of adults were released in the cage (36 × 26 × 33 cm) with one potted broccoli plant. After 24 hours, the numbers of eggs laid on the upper and under surfaces of leaves and on the stem were recorded. The female adults laid 36.1, 45.2 and 18.7% of eggs on the upper and under surfaces of leaf, and stem, respectively. The water sprinkling (about 60 mm/min precipitation) was done for 1 min from 1 m above broccoli plants on which eggs of DBM were laid. Three sprinklings, 1, 2 and 3 times each day on 1, 2 and 3 days, respectively, were done and the number of eggs on the plants was recorded before and after sprinkling. The rate of wash-off of eggs under the water sprinkling is given in Table 4. On average, 29.6% of eggs were washed off during one sprinkling. The percentage of wash-off was highest (47.4%) on the upper surface of the leaf followed by 25.8% on the under surface. The percentage of wash-off increased with the number of sprinklings. After three sprinklings, 72.4% of the eggs on the upper surface were washed off. However, 20% of the eggs also fell after 3 days even without the sprinkling. This suggests that the eggs of DBM are laid innately in an easily detachable manner. Therefore, even a slight shock by rainfall may cause the eggs to fall.

The water sprinkling was also conducted on eight plants on which 30 DBM larvae of different stages were inoculated. The effects of water sprinkling on wash-off of larvae and pupated individuals were observed. The percentage of wash-off was 24.0, 24.2, 20.7, 11.6 and 5.6 in the 1st, 2nd, 3rd, 4th instar and pupae, respectively.

Table 4. The wash-off of eggs of DBM by water sprinkling (Wakisaka et al., 1991).

Water sprinkling[a]	Part of plant	Eggs (%) washed off at sprinkling-		
		1st	2nd	3rd
Treated	Upper surface	47.4	67.6	72.4
	Under surface	25.8	37.4	50.8
	Stem	4.5	3.5	12.1
	Number of eggs	29.6	41.0	52.0
Untreated	Upper surface	22.6	7.9	22.7
	Under surface	9.7	12.9	17.9
	Stem	19.1	16.0	20.0
	Number of eggs	16.5	11.4	19.9

[a]The amount of water was equivalent to 60 mm percipitation.

Effects of Stearic Acid Leaf on Egg Wash-off

Kimura (1987) reported that the amount of wax bloom on the leaf of cabbage was 36 $\mu g/cm^2$. We considered that the amount of wax bloom on broccoli is not so different from that of cabbage. Stearic acid was used as an alternative material to the wax. Seven kinds of leafdisks (2 cm in diam) of broccoli were prepared as follows: Untreated leafdisk (TCO), 0.025 ml of acetone were applied on the intact leafdisk (TA1). The leafdisk was washed with absorbent cotton containing acetone (TA2), 0.05, 0.1, 0.2 and 0.3 g of stearic acid were diluted with 26 ml of acetone and 0.025 ml of this solution was applied on the leafdisks (T0.5, T1, T2 and T3). Thirty pairs of adults were introduced into the cage containing five leafdisks of each treatment placed in a petri dish and were made to lay eggs on the disks for 24 hours. The leafdisks were then placed on the round plate (9 cm diam.), and 150 ml of water was sprinkled from a height of 30 cm for 1 min while slowly rotating the plate. The number of eggs on the leafdisk was recorded before and after the water sprinkling.

The relationship between the amount of stearic acid layer on the leafdisk and the percentage of egg falling under the water sprinkling is given in Table 5. The percentage of egg washed off was lowest on TA2 and highest on T3. The difference was significant between TCO and all other treatments except for T1 (χ^2-test). However, the increase in the amount of stearic acid of more than a half of the natural amount of wax layer did not increase the percentage of eggs wash-off.

Table 5. Relationship between the amount of stearic acid on broccoli leafdisk and percentage of wash-off of eggs by water sprinkling.

Treatment[a]	No. eggs	Wash-off of eggs[b]	
		No.	%
TCO	1115	287	25.7
TA1	344	35	10.2**[c]
TA2	879	49	5.6**
T0.5	762	238	31.2*
T1	1040	306	29.4n.s.
T2	1197	362	30.2*
T3	1275	399	31.3**

[a]TCO, untreated leafdisk; TA1, only acetone; TA2, washed wax with acetone; T0.5-T3, stearic acid (0.5-3 times of amount of natural wax). [b]The amount of water was equivalent to 20 mm precipitation. [c]Figures with asterisks differ significantly from TC0 at 5% level (*), 1% level (**) and not significant (n.s.) by χ^2-test.

Temperature and Host Plant Effect on Survival and Reproduction

Immature stages of DBM were reared individually at 25.0, 27.0, 28.5, 30.5, and 33.0°C under 16L:8D and 90% RH. Larvae were fed 4-week-old broccoli leaf. A pair of adults were caged and provided with 10% honey solution and the pieces of cabbage leaves. The developmental period of immature stages, and longevity and fecundity of adults were measured by daily observation.

Influence of host plants on the development of larvae and the performance of adults was investigated under laboratory conditions. Four crops were used: broccoli (cv. Ryokurei), young and mature cabbage (*B. oleracea* var. *capitata* cv. Green ball) and Chinese cabbage (*B. campestris* ssp *pekinensis*, cv. Seikai), and one wild crucifer, *Capsella bursa-pastoris*. All host plants were grown in the greenhouse. Larvae were reared individually and adults were paired at 25°C under 16L:8D and 90% RH.

The percentage of egg hatching was 85-92% at all temperatures. The developmental period from egg to emergence of adults was 15.2, 14.9, 13.8, 12.8, and 14.8 days at 25.0, 27.0, 28.5,

30.5 and 33.0°C, respectively. The developmental period shortened with increased temperature, however it was delayed for all stages at 33°C. The percentage of adult emergence was 90.3, 75.8, 62.9, 87.5, and 19.2 in the increasing order of temperatures up to 33°C.

The developmental period from egg to emergence of adults was 15.2, 16.8, 18.7, 15.8, and 16.5 days and the adult emergence was 90.3, 65.7, 59.4, 69.4, and 22.4% when larvae fed on broccoli, young cabbage, matured cabbage, Chinese cabbage, and *C. bursa-pastoris* at 25.0°C, respectively.

The longevity of adults, preovipositional period and fecundity of females are shown in Table 6. The longevity of females is shortened with increasing temperature. The preovipositional period was also shortened with increasing temperature, however it was delayed at 33°C. The fecundity was highest, 241 eggs/female, at 25°C, followed by 27.0, 30.5, 28.5, and 33.0°C in decreasing order. It was lowest, 52 eggs/female, at 33°C.

Both sexes of DBM lived longest when fed on young cabbage leaves. The preovipositional period of DBM fed on broccoli was shortest, while DBM that fed on the weed *C. bursa-pastoris* it was longest. The fecundity of DBM fed on broccoli, Chinese cabbage and young cabbage was significantly higher than those fed on mature leaves of cabbage and *C. bursa-pastoris* ($P<0.05$).

Table 6. Biotic performances of adults at different temperatures and host plant conditions.

	Sex	Longevity[a] (days) $\bar{X}\pm SE$	Preovipositional period (days) $\bar{X}\pm SE$	Fecundity (No. eggs/female) $\bar{X}\pm SE$
Temperature(°C)				
25.0	Male	12.21 ± 1.13b	–	–
	Female	11.08 ± 0.54c	0.33 ± 0.19a	241.08 ± 19.57b
27.0	Male	8.70 ± 1.48ab	–	–
	Female	8.75 ± 0.72c	0.40 ± 0.16a	200.90 ± 17.28b
28.5	Male	11.50 ± 3.76b	–	–
	Female	9.07 ± 0.97c	0.00 ± 0.00a	192.40 ± 37.35b
30.5	Male	9.58 ± 0.15ab	–	–
	Female	9.38 ± 0.60c	0.08 ± 0.08a	202.92 ± 19.32b
33.0	Male	5.17 ± 1.01a	–	–
	Female	6.33 ± 0.33c	2.00 ± 1.15b	52.00 ± 4.51a
Host plants				
Broccoli	Male	12.21 ± 1.13a	–	–
	Female	11.08 ± 0.54d	0.33 ± 0.19a	241.08 ± 19.57b
Cabbage: young leaves	Male	29.50 ± 2.53c	–	–
	Female	20.10 ± 1.62e	0.57 ± 0.27a	257.80 ± 20.22b
Cabbage: mature leaves	Male	14.33 ± 1.67a	–	–
	Female	11.75 ± 1.16d	1.33 ± 0.33b	146.83 ± 17.06a
Chinese cabbage	Male	12.00 ± 0.96a	–	–
	Female	11.96 ± 0.53d	0.71 ± 0.21ab	246.71 ± 14.58b
Wild crucifer[b]	Male	21.29 ± 1.43b	–	–
	Female	17.90 ± 1.19e	2.83 ± 0.54c	138.00 ± 13.56a

[a]Statistical test was done among the temperature and host plant conditions. For longevity, the test was done for each sex. The same letter means not significantly different at 5% level by Duncan's multiple range test. [b]*Capsella bursa-pastoris*.

The demographic parameter values were estimated using the data of the hatching percentage, the developmental period from egg to adult emergence, the survival rate (lx), and daily fecundity (mx) of females under different temperatures and different host plants. The sex ratio was assumed to be 1:1. The estimated values are shown in Table 7. The net reproductive rate (R_o) was highest at 25°C and lowest at 33°C. The generation time (G) was shortest at 30.5°C. The intrinsic rate of increase (r_m) increased as temperatures rose, however it was lowest at 33°C.

The net reproductive rate (R_o) was highest for DBM which fed on broccoli; it was lowest when DBM fed on the weed. The decreasing order in the crops was young cabbage, Chinese cabbage and mature cabbage. The generation time (G) was about 19 days on broccoli and Chinese cabbage, 23 days on mature leaves of cabbage, 25 days on young leaves of cabbage, and 24 days on *C. bursa-pastoris*. The value of r_m was highest on broccoli and lowest on *C. bursa-pastoris*.

Table 7. Demographic parameters under different temperatures and host plant conditions.

	Net reproductive rate (R_0)	Generation time (G) (days)	Intrinsic rate of increase (r_m)
Temperatuare (°C)			
25.0	197.03	19.02	0.2778
27.0	140.23	17.80	0.2777
28.5	98.98	16.35	0.2810
30.5	158.99	15.51	0.3268
33.0	4.88	18.62	0.0851
Host plant			
Broccoli	197.03	19.02	0.2778
Cabbage: young leaves	154.71	21.93	0.2298
Cabbage: matuare leaves	80.18	24.91	0.1760
Chinese cabbage	153.65	19.43	0.2592
Wild crucifer[a]	27.52	24.35	0.1362

[a] *Capsella bursa-pastoris*.

Discussion

Life tables of DBM have been reported by Harcourt (1963), Iga (1985) and Sivapragasam et al. (1988). In the present study, the mortality rate of eggs and young larvae was high and the main mortality factor was disappearance from unknown causes. The natural enemies were an important factor in mortality of the old larvae and pupae in summer and autumn. Sivapragasam et al. (1988) reported that 38% of egg and larval mortality was due to wash-off of eggs through direct impact of rainfall. Harcourt (1963) and Nakagome and Kato (1974) also remarked on the effect of rains on young larvae. Talekar et al. (1986) reduced the infestation of DBM using overhead sprinkler irrigation, because this system affects adult activity and oviposition. In the present study, when the experimental plots in the field were shielded from precipitation (Plot C and R), the survival rates of eggs and young larvae were higher than those in the plots (plots G and A) where the effect of precipitation was not excluded (Table 1-3).

The eggs of DBM on host plants, especially on leaves, were washed off easily under the water sprinkling (Table 4). Most DBM eggs are not laid on the stem but on the surface of leaves. Larvae also were washed off by the sprinkling. The results suggest that the direct impact of rainfall is an important factor in the disappearance of eggs and larvae.

The washing-off of eggs is considered to be influenced by the wax bloom on leaves. The experiment using stearic acid as an alternative material to wax, showed that the proportion of egg fallings was low when the leafdisk was not coated with stearic acid. Eckenrode et al. (1986) reported that alteration of wax condition by ether dips or brushing increased the oviposition

of DBM. Uematsu and Sakanoshita (1989) also reported similar results. The wax layer on the leaf surface is thought to suppress oviposition. The mortality rate of young larvae is related to the amount of rainfall (Harcourt 1986). In our study larvae were also washed off by sprinkling. When larvae were reared under high humidity (about 100%), the mortality rate of larvae was 70%, whereas it was 30% under less than 90% RH (Wakisaka, unpublished data). High humidity also affects the survival of larvae.

Koshihara (1986) suggested that the seasonal fluctuations of DBM populations are similar in varied locations in Japan. His suggestion is supported by Yamashita (1963), Nakagome and Kato (1974), Yamada (1977), Iga (1985) and Iwata (1986). These studies showed DBM populations usually peak around May, and drastically decrease in summer. Population densities increase again in the autumn. Our life tables show that survival rates in the early and mid summer are extremely low. On the other hand, the rates in autumn become high. Low survival rate in the summer is probably due to high temperatures and fewer and lower quality host plants.

Umeya and Yamada (1973a, b), Nakagome and Kato (1975), Yamada and Kawasaki (1983) and Sarnthoy et al. (1989) reported that high temperatures adversely affected the development of DBM. The rearing experiment in our study also shows that the developed period delays and emergence rate become low at 33°C. Furthermore the fecundity of females was low at 33°C (Table 6). High temperatures may suppress the behavior of oviposition and copulation (Yanagida and Sakanoshita 1974).

The decrease in the acreage of vegetable cultivation may also affect the population abundance of DBM in the summer. Under such conditions, DBM must depend on cruciferous weeds. Our results show that *C. bursa-pastoris* is considerably less suitable for DBM (Table 7), although wild hosts are not always less suitable (Yamada 1983).

The DBM which does not have diapause can develop slowly even in the winter season. The delayed developmental period in the winter causes high mortality (Wakisaka, unpublished data), and population density does not increase. In spring, development becomes faster, and cultivation area of cruciferous crops increases gradually. The major parasites are still not active, therefore the population density increases rapidly in the spring. In the rainy season of the early summer, mortalities from wash-off of eggs and the drowning of young larvae become high. In mid summer, upland ground surface temperature becomes considerably higher than the average temperature, and adverse physiological effects suppress the population. The parasites also become active in this season. Furthermore the area of cruciferous crops decreases and the habitat of DBM is limited. It is considered that most DBM must feed on wild cruciferous weeds which are generally less suitable for host plants than cruciferous crops. All these factors tend to decrease the population density in summer. In autumn, the area of cruciferous crops increases again, and the activity of parasites becomes low. Therefore, the survival and reproduction rates become high. This is a possible explanation for the seasonal prevalence of DBM in Japan.

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2

Natural Mortality of Diamondback Moth in Coastal South Carolina

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Abstract

Mortality of the diamondback moth, *Plutella xylostella* was assessed in field plots of collards using exclusion cages and a pyrethroid insecticide.Numbers of insects in untreated plots were kept below economically damaging levels by indigenous arthropod predators and the parasitoid *Diadegma insulare* (Cress.). Parasitism of DBM by *D. insulare* reached 95% in plots without the pyrethroid treatment. Predators accounted for up to 72% of larval mortality. The pyrethroid treatment caused diamondback moth resurgence due to reduction in natural enemy densities. Major predators were identified and laboratory studies showed that the spider *Pardosa milvina* (Hentz) consumed about one larva/day. This predator was the most active ground-dwelling species as determined by pitfall trapping, and its numbers were significantly reduced by pyrethroid insecticide applications. Simulated and natural rainfall did not cause significant diamondback moth larval mortality under our test conditions.

Introduction

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera:Yponomeutidae), remains the most serious insect problem on crucifers worldwide. In some areas of tropical Asia cocktail mixtures of insecticides are applied every other day for this pest without satisfactory control (M. Shepard, personal observations, Philippines). It is clear that the misuse of insecticides has exacerbated problems with DBM (Ooi and Sudderuddin 1978) and development of more ecologically-based management strategies has been slow in coming and difficult to implement on a large scale.Ullyett (1947) demonstrated that insecticides caused DBM populations to increase, and emphasized the negative effects of chemicals on natural biological control agents. In addition to chemical applications, many farmers now incorporate *Bacillus thuringiensis* Berliner into their control programs. Recent reports of DBM resistance to *B. thuringiensis* (Tabashnik et al. 1990) underlines the importance of developing management systems that employ a multifaceted approach with biological control as the core (Ooi 1990).

There are many examples of suppression of DBM populations using imported parasitoids (Goodwin 1979; Ooi 1990; Ooi and Lim 1989; Cock 1983; Sastrosiswojo and Sastrodihardjo 1986). Waterhouse and Norris (1987) selected DBM as one of several species as a likely candidate for successful classical biological control. Their major reason for suggesting this species was that biological control has already been achieved in several parts of the world with parasitoids. The major species used in these efforts in Asia include *Diadegma semiclausum* Horstmann, *Diadromus collaris* Gravenhorst, and *Cotesia plutellae* Kurdjumov.

Diadegma insulare (Cress.) is the most important parasitoid of DBM in North America (Latheef and Irwin 1983; Pimentel 1961; Oatman and Platner 1969; Harcourt 1960, 1963, 1986; Lasota and Kok 1986; Horn 1987). A few other parasitoid species (*Microplitis plutellae* Muesbeck,

Tetrastichus sokolowskii Kurdjumov, *Cotesia plutellae* Kurdjumov, and *Spilochalcis albifrons*) have been reported to contribute to a lesser extent to DBM mortality (Parker 1971; Horn 1987; Ru and Workman 1979).

Surprisingly little attention has been given to assessing the role of predators in DBM management. Oatman and Platner (1969) listed syrphid larvae, coccinellids, *Geocoris*, *Orius*, nabids, and chrysopids as possible predators of DBM but quantitative assessments of their impact were not made. Likewise, a number of predatory arthropods were reported as important sources of DBM mortality in South Africa (Ullyett 1947). These included staphylinids, wasps of the genus *Polistes*, syrphids, chrysopids, hemerobiids, and anthocorids. Of these, syrphids and anthocorids were thought to be most important. Many of these predators were attracted initially to aphids and switched to DBM as aphid populations declined. Ullyett (1947) followed DBM populations through several periods and recorded total mortality between 83 and 92%. Of this, 23% was attributed to predation.

Suppression of DBM by field application of a granulosis virus has been reported (Wang and Rose 1978) and epizootics of the fungal pathogen *Zooptera radicans* (Brefeld) (= *Entomophthora sphaerosperma*) are considered important in some areas, e.g. South Africa (Ullyett 1947), Malaysia (Ooi 1979, 1981), New Zealand (Kelsey 1965), in North Carolina (K. Sorenson, North Carolina State University, personal communication) and Florida (G. Leibee, University of Florida, personal communication) but control by indigenous fungal pathogens has been sporadic and largely dependent upon rainfall (high humidity).Rainfall also has contributed directly to DBM mortality in the field by dislodging and drowning young DBM larvae (Hardy 1938; Harcourt 1963; Talekar et al. 1986).However, Ullyett (1947) did not consider the direct impact of rainfall as a significant mortality factor.

The objective of our study was to identify major sources of mortality of DBM eggs and larvae in collards in coastal South Carolina.

Materials and Methods

Seasonal abundance of DBM and incidence of parasitism

A 0.73 ha collard field on the Coastal Research and Education Center farm, Charleston, South Carolina, was planted to 'Vates' variety collards on 27 February 1990, and thinned to 46 cm plant spacings on 17 April 1990. The field was divided into 12 plots, 0.025 ha each, and weekly sampling was carried out by inspecting 25 plants from each plot. DBM larvae, pupae and *D. insulare* pupae were counted. Plots were treated with either Javelin (*B. thuringiensis kurstaki*) at 0.56 kg/ha or the pyrethroid Asana-XL at 0.04 kg ai/ha. Control plots were left untreated. There were four replications. Treatments were applied on 25 April, 2 May, 23 May, 30 May, 6 June and 13 June 1990.

In addition to in-field counts, weekly collections of DBM larvae were made from five separate plants in each plot from 24 April to 18 June 1990.Larvae were placed on artificial diet and held in a rearing room at 26°C, 70±10% RH and a photoperiod of LD 12:12. Numbers of adult DBM and parasitoids that emerged were recorded.

Activity of ground-dwelling predators

Ground-dwelling predators were collected weekly using two pitfall traps in each plot from 11 May to 19 June 1990. Traps consisted of plastic DG8 Solo cups (11 cm diam x 4 cm depth) with tin covers (20 x 22 cm) suspended 2 cm above the cups to keep out rain. Ethylene glycol was placed in each trap to a depth of 1 cm to kill and preserve the arthropods.

Laboratory and field cage studies

Predation by *Pardosa milvina* in laboratory cages. Predation by *Pardosa milvina* (Hentz) on DBM larvae was determined in the laboratory using spiders collected from collard fields

at the Coastal Research and Education Center farm, Charleston, South Carolina. Cage tests were carried out at Clemson University, Clemson, South Carolina.

Four-week-old collard plants were potted and caged individually using cylindrical plastic cages (9 cm diam × 19 cm high). First and second instar DBM were categorized as small and third and fourth instars were considered large. Small and large larvae were introduced into cages at density levels of 1, 2, 4 and 8 larvae/cage. Larvae were allowed to settle on the plants for about 2 hours before spiders were introduced. Spiders were starved for 5 days before the test began. Numbers of larvae consumed were recorded daily for 7 days. Larval density per cage was maintained by daily replacement. Also, larvae that molted to third instars in the small larvae treatments and those that pupated in the large larvae treatments were replaced. Partially consumed or dead larvae were counted as consumed. Each treatment was replicated six times except for controls (cages with no spiders) which were replicated three times. ANOVA followed by Duncan's multiple range test ($P<0.05$) was used to analyze data.

Predation on eggs. Egg predation studies were initiated by placing 4-6 colony-reared male and female DBM adults into small clip cages (DeBach and Huffaker 1971) on randomly selected pairs of plants in untreated control plots. These were left overnight and the clip cages were removed and eggs counted the next day. Clip cage location was marked on the plant in waterproof black ink to facilitate finding the eggs. Nylon mesh cages were then placed over one plant. The other plant was exposed to predators for 1 day and missing eggs were recorded. Two sets of tests were conducted: one using five pairs of cages (10-11 July) and the other using eleven pairs (20-21 July).

Larval predation. Field cage studies of predation on second to third instars were carried out in the untreated control plots on 40 randomly selected pairs of plants. Predators were removed from each plant and 10 or 15 DBM larvae were placed on both plants. A nylon mesh cage was then placed over one plant. Each cage was approximately 36 cm wide × 50 cm high supported by bent wires with ends pushed into the soil. The base of the nylon mesh cage cover was sealed on all sides with soil to prevent the entry of predators. Larvae were counted from all plants after 4 days.Two separate tests were carried out: one on 26-30 June 1990 and the other 6-10 July 1990.Control mortality (missing larvae) from the caged plants was accounted for using Abbott's formula (Abbott 1925).

Influence of rainfall on DBM mortality

Actual rainfall. Ten pairs of collard plants were selected from untreated control plots prior to expected thunderstorms and carefully cleaned of all arthropods. Second and third instar colony-reared larvae were placed on the plants and allowed to settle in before rainfall occurred. Just prior to the thunderstorm, a plastic bag was placed over one plant of the pair while the other plant was left exposed.The plastic cover was supported by wires described earlier for the larval predation study. At the end of the thunderstorm, covers were removed and larvae were counted. Rainfall was measured with a standard rain gauge. Student's t-test was used to compare means ($P<0.05$).

Simulated rainfall. 'Vates' variety collards were established in 1-l containers in the greenhouse with temperatures ranging from 18 to 27°C. Tanglefoot was applied to each container to exclude predators. Eight field-collected second to fourth instar DBM were placed on each plant for a minimum of 18 hours before simulated rainfall was applied. Most of the larvae moved to the undersides of the leaves and began feeding.

Simulated rainfall was achieved using a Spraying Systems Fulljet 1/4 HH 14.5 square cone spray nozzle at 0.55 kg/cm^2. The nozzle was placed 2.44 m above the top of the plants to allow water droplets to achieve terminal velocity (Shelton et al. 1985). The nozzle produced a drop size of 1140-4300 μ which is about the same size as that produced in a normal thunderstorm

in South Carolina. Rainfall rate was 16.8 cm/hour and treatments consisted of simulated rainfall for 0, 20, 40, and 60 min.

Nine collard plants about 30 cm wide and 25 cm high were spaced at 30 cm intervals beneath the nozzle. Three replications were carried out and data were analyzed using ANOVA followed by Duncan's multiple range test ($P<0.05$).

Results

Seasonal abundance of DBM and incidence of parasitism

In general, natural enemies kept DBM populations below economically important levels throughout the growing season. Mean numbers of DBM larvae and pupae per plant are shown in Fig. 1 and 2, respectively. DBM population levels in *B. thuringiensis*-treated plots were approximately the same as those from untreated ones, but DBM numbers increased in pyrethroid-treated plots after 5 weeks and reached a peak of about 2 larvae/plant before declining. Parasitism by *D. insulare* reached a peak of over 90% in untreated plots. It is likely that resurgence in DBM numbers in the pyrethroid-treated plots was due to destruction of natural enemies by this chemical. Highest numbers of *D. insulare* pupae were found in the untreated plots (Fig. 3), and because of the upsurge in larval density in the pyrethroid-treated plots, the parasitoid moved into these plots and higher numbers of *D. insulare* pupae were subsequently found there during the last two sampling periods. It is likely that action by the parasitoid and not the chemical caused DBM populations to decline in these plots (Fig. 1 and 2, respectively).

Season-long collections of 1192 DBM larvae reared on artificial diet revealed that parasitoids emerged from 41%, DBM adults from 5%, and 54% died from unknown causes. More than 95% of all parasitoid species were *D. insulare*. High mortality of DBM in field collections was due to handling during collection and problems with rearing.

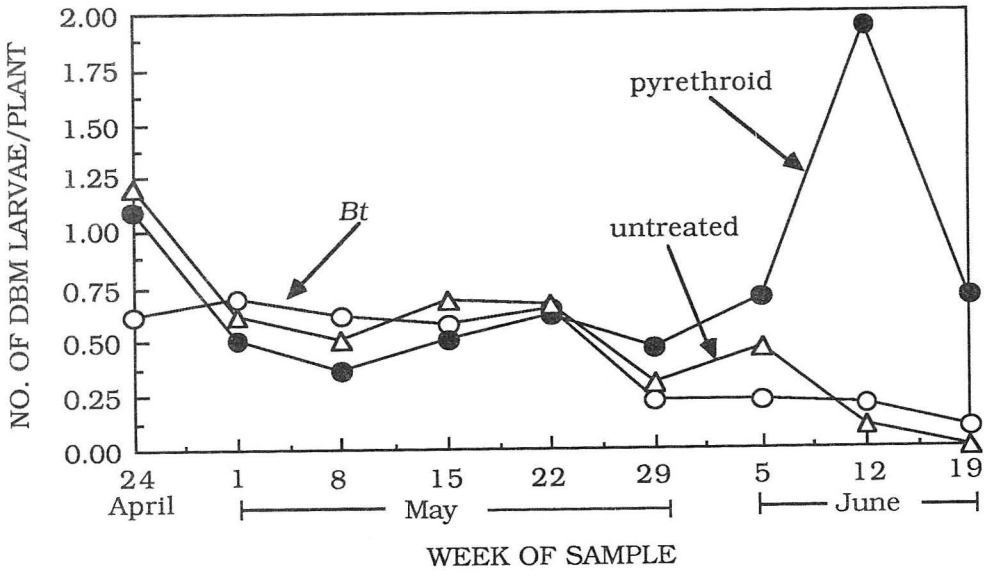

Fig. 1. Seasonal abundance of DBM larvae from untreated collard plots and those treated with a pyrethroid and *Bacillus thuringiensis*. Charleston, South Carolina, 1990.

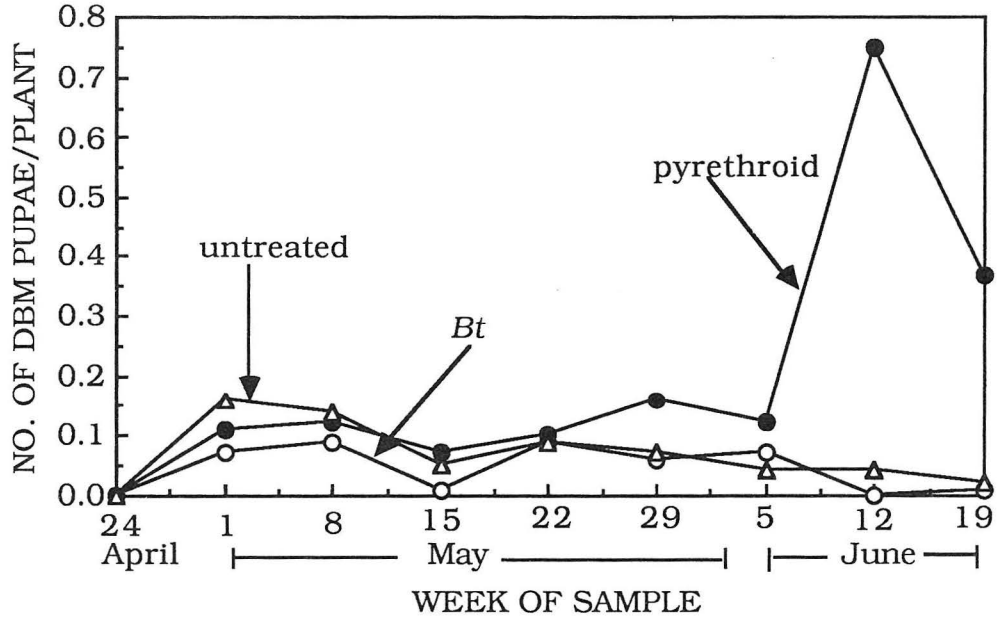

Fig. 2. Seasonal abundance of DBM pupae from untreated collard plots and those treated with a pyrethroid and *Bacillus thuringiensis*. Charleston, South Carolina, 1990.

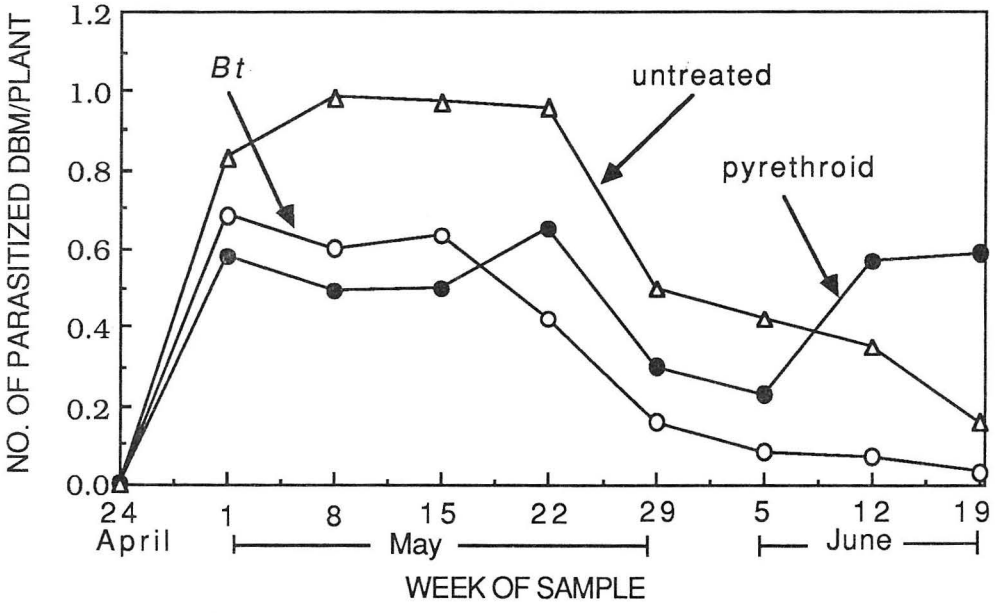

Fig. 3. Seasonal abundance of *Diadegma insulare* pupae per plant in untreated collard plots and those treated with a pyrethroid and *Bacillus thuringiensis*. Charleston, South Carolina, 1990.

Activity of ground-dwelling predators

By far the most numerous ground-dwelling predator determined by pitfall trap collections was the lycosid *Pardosa milvina*. The seasonal activity of this spider is shown in Fig. 4. Mean

numbers of *P. milvina* from the *B. thuringiensis*-treated, untreated and pyrethroid-treated plots were 8, 7, and 3 spiders/trap, respectively. Numbers of spiders were significantly ($P < 0.05$) lower in the pyrethroid-treated plots. The *P. milvina* population peaked on 21 May 1990 and gradually declined throughout the remainder of the season although there was a gradual increase during the last 2 weeks in the untreated plots. Other major predators that were commonly encountered in the collard plots are listed in Table 1.

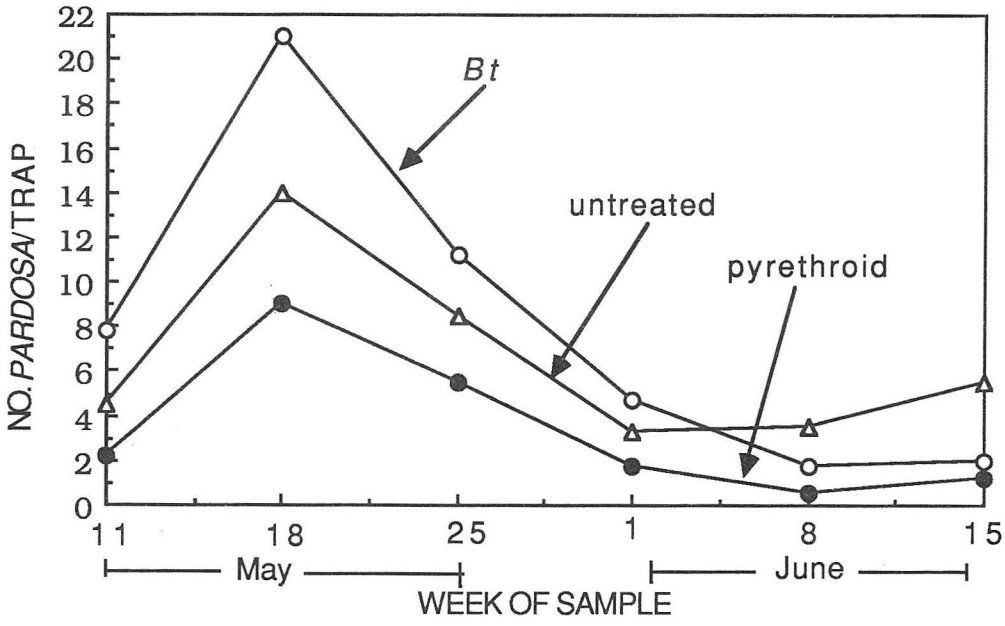

Fig. 4. Numbers of *Pardosa milvina* per trap in untreated collard plots and those treated with a pyrethroid and *Bacillus thuringiensis*. Charleston, South Carolina, 1990.

Table 1. Common predators in South Carolina collard fields.

ARACHNIDA

Lycosidae
- *Pardosa milvina* (Hentz)
- *Pardosa pauxilla* Montgomery
- *Pardosa delicatula* Gertsch & Wallace (New Record)

Erigonidae
- *Eperigone fradeorum* (Berland)

Linyphiidae
- *Florinda coccinea* (Hentz)

INSECTA

Formicidae
- *Solenopsis invicta*
- Unidentified spp.

Coccinellidae
- *Coccinella septempunctata* L.
- *Hippodamia convergens* Guerin-Meneville
- *Coleomegilla maculata* (DeGeer)
- *Scymnus* spp.

Syrphidae
- Unidentified spp.

Carabidae
- *Calosoma sayi*

Lygaeidae
- *Geocoris punctipes* (Say)
- *Geocoris uliginosus* (Say)

Pentatomidae
- *Podisus maculiventris* (Say)

Nabidae
- *Nabis americoferus* Carayon

Vespidae
- *Polistes* spp.

Hemerobiidae

Chrysopidae

Labiduridae

Reduviidae

Anthocoridae

Laboratory and field cage studies

Predation by *P. milvina* in laboratory cages. Numbers of small and large DBM larvae consumed by *P. milvina* in laboratory cages are shown in Fig. 5A and 5B, respectively. At all density levels, consumption increased with time and prey density. There was no significant difference between consumption of small and large larvae. In general, each *P. milvina* consumed about 0.5-1 larva/day at the highest host density (eight).

It is not possible to extrapolate these results to the field. However, high activity levels of *P. milvina* in the collard plots as detected by pitfall traps and results from the field cage studies which showed up to 72% mortality of DBM larvae by indigenous predators, provide strong evidence that this spider species may be an important source of DBM mortality.

Predation on eggs. Numbers of eggs removed by predators were highly variable. This may be due to the artificially clumped food source. None of the eggs were missing after 1 day from the tests using five pairs of clip cages. On the other hand, percent missing eggs ranged from 0 to 100% ($\bar{x}$ = 42%) in the second test (n = 11). The implications here are unclear and further testing is necessary using the appropriate egg distribution pattern.

Predation on larvae in the field. Average percent DBM larvae missing from uncaged plants (n = 40) in test one was 60% after correcting for missing larvae in cages. In test two (n = 40), 72% of the larvae were missing from the uncaged plants due to the action of indigenous predators.

Larval mortality due to actual rainfall

Influence of rainfall on DBM mortality. Second and third instar DBM larvae were exposed to normal afternoon thunderstorm activity producing 0.89 cm of rain in test one and 3.13 cm of rain in test two. However, there was no evidence to suggest that rainfall washed small DBM larvae from plants (e.g., counts of larvae on covered plants were not significantly different from those on exposed plants).

Field observations by the authors during heavy rainfall revealed that larvae were not affected by natural rainfall under conditions of our test.

Simulated rainfall. There was no significant difference in numbers of larvae from control plants and those subjected to simulated rainfall.These results are inconsistent with reports of loss or mortality of DBM larvae due to rainfall (Hardy 1938; Harcourt 1963; Talekar et al. 1986). However, these experiments were conducted on collard plants under controlled conditions with no wind and at optimum temperature. This does not rule out the possibility of reduction of DBM populations due to a combination of environmental factors or the disruption of adult flight, mating, or ovipositing (Talekar et al. 1986). In addition, collard plant hosts may have provided more protection from being dislodged by rainfall than would cabbage or some other cruciferous crop.

Discussion

The population density of DBM rarely reached levels that would be considered economically important except in plots treated with the pyrethroid insecticide.Our preliminary evidence shows the parasitoid *D. insulare* and indigenous communities of arthropod predators are the major mortality factors impacting mainly on DBM larvae. Indigenous pathogens were not important. Data from laboratory studies of predation by *P. milvina* suggest that this spider is an important member of the predator complex, and its populations were most active according to pitfall trap sampling. Nemoto (1986) used a precipitin test to show that lycosid spiders were an important

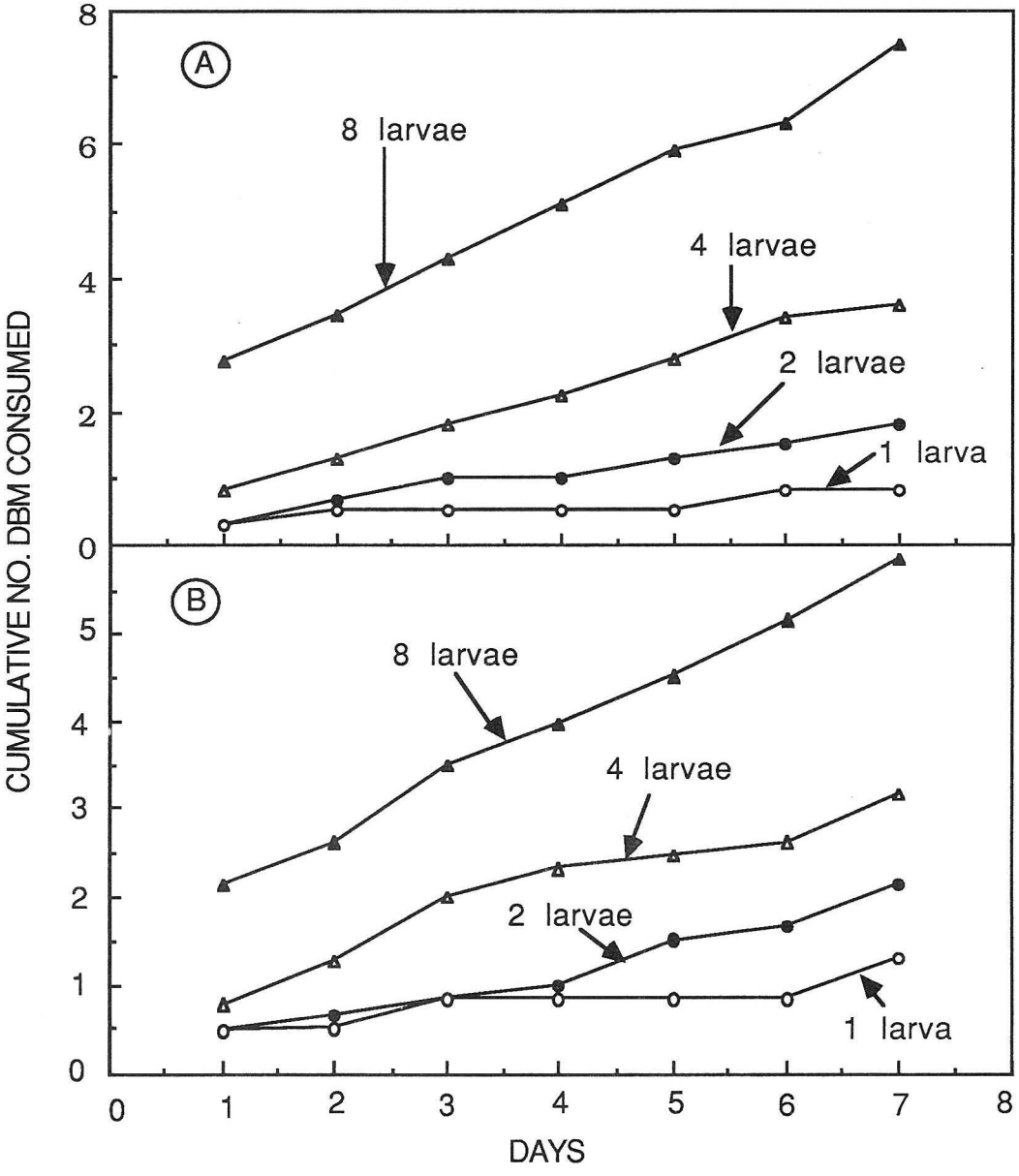

Fig. 5. Cumulative numbers of 'small' (A) and 'large' (B) DBM larvae consumed by *Pardosa milvina* in laboratory cages over collard plants. Clemson, South Carolina, 1990.

source of mortality for third and fourth instar DBM. Under conditions of our tests, neither natural nor simulated rainfall caused significant DBM mortality.

It is clear that in some areas of the world, the rich complex of natural enemies (either introduced or indigenous) keep DBM populations in check unless chemical insecticides are applied. We believe that this is the case for coastal South Carolina.

Further research is needed to quantify the impact of arthropod natural enemies more clearly and to develop a management program for DBM that incorporates this information into IPM decisions.

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3

Effects of Age and Body Size on the Mating Success of Diamondback Moth

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Abstract

When female diamondback moth, *Plutella xylostella* (L.), were individually placed with a male in a glass tube, some (20-35%) laid only unfertilized eggs. To study this phenomenon, and in order to clarify the effect of age on mating, each of the 0-8-day-old females was placed with each of the 0-8-day-old males in a glass tube for 2 days. Successful copulation was determined by checking the embryonic development of the eggs the female laid. The number of females laying eggs increased from 65% in the 0-day-old age-group to 93.5% in the 8-day-old group. However, the number of females laying fertile eggs was unchanged at about 50%. The proportion of mating success was also independent of the age of the male. The effect of body size on mating success was also examined. Each of the females (3-8 mg) was placed with a male (size varied). When the difference in body weight between the paired insects was 2 mg or less, 79.8-88.1% of the females successfully mated. When the difference was 3 mg or more, the mating success was reduced to 63.5%. This suggests that large intersexual differences in body weight has some ill-effects on the mating behavior of the pair.

Introduction

In the course of studies on the fecundity of diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), the author found that some females laid only unfertilized eggs. Yamada and Umeya (1972) also observed this phenomenon, and found that some females laid only unfertilized eggs even when two males were placed with the female in a container. Many workers (Sakanoshita and Yanagita 1972; Ohira 1979; Maa et al. 1985) have studied mating behavior of DBM, but the effects of age and body size on mating success are still unknown. This paper describes the results of laboratory experiments on this aspect of DBM mating.

Materials and Methods

Effects of Age on Mating Success

A large number of pupae were continuously collected from cabbage fields in Miyazaki, in the southern part of Japan, between January and March 1990. They were individually placed in a glass tube (15 × 150 mm) and reared at 20°C, 14L:10D until the day of testing. After emergence adults were provided with water. Each of the 0-, 2-, 4-, 6-, and 8-day-old females was paired with 0-, 2-, 4-, 6-, and 8-day-old males in a glass tube (30 × 200 mm). A piece of Japanese radish leaf was placed in the glass tube because DBM laid eggs on it more frequently than on other cruciferous plants such as cabbage or broccoli (Uematsu and Sakanoshita 1989). The glass tubes were placed in the chamber for a period of 2 days. Successful copulation was determined by checking the embryonic development of the eggs laid. Forty pairs were examined in each of the combinations.

Effects of Body Size on Mating Success

DBM pupae were collected in a cabbage field in Miyazaki in May 1989. The progenies were reared on the Japanese radish leaves at 15-25°C to obtain insects of various sizes. Before pupae were individually placed in the glass tubes, they were weighed and separated into five size groups of 3.0-3.9, 4.0-4.9, 5.0-5.9, 6.0-6.9, and 7.0-7.9 mg. Pupal weight was used as an indicator of the adult size. Until the day of test, they were reared under the conditions above. Each of the females of five groups was paired with various-sized males for a period of 2 days. Although the ages of moths were not strictly arranged, they ranged from 0 to 5 days. I examined 855 pairs (Table 1). Successful copulation was determined by checking the embryonic development of the eggs laid.

Table 1. Size class of DBM and number of pairs tested in each of the combinations.

Size class of female (mg)	Size class of male (mg)				Total
	3.0-3.9	4.0-4.9	5.0-5.9	6.0-6.9	
3.0-3.9	20	36	16	5	77
4.0-4.9	78	79	80	11	248
5.0-5.9	64	80	80	21	245
6.0-6.9	15	80	79	32	206
7.0-7.9	6	35	21	17	79
Total	183	310	276	86	855

Results and Discussion

Effects of Age on Mating Success

The number of pairs that laid eggs varied from 22 to 40, averaging 32.5 (Table 2). This was not correlated to the age of the male, but was significantly correlated to that of the female ($P<0.01$). This indicates that older females lay eggs more easily than younger females when they are paired with males. Since virgin DBM females also lay eggs (Uematsu, unpublished data), the values in Table 2 do not mean the number of successful matings. Of course, in the cases where females laid no eggs, it was impossible to determine whether they had mated or not. Therefore, the data on female egg-laying were used to analyze the effect of age on mating. The proportion of females laying fertile eggs to females laying eggs irrespective of their fertility is given in Table 3. The proportion was independent of the age of the male, but dependent on that of the female ($P<0.05$). The youngest age-group of females shows the highest value of mean, 0.800, though the other age-groups show no difference in the mean values. Two combinations, 2-day-old-female:6-day-old-male and 6-day-old-female:0-day-old-male, gave very small values. The reason for this is unknown. However, these values were not responsible for the significance, because reanalysis of the data excluding these combinations also showed highly significant differences ($P<0.001$). Therefore, it is concluded that the eggs laid by younger females have a higher possibility of being fertile than those laid by older ones. However, it does not always follow that the younger females are more easily fertilized than the older ones, because the number of females laying fertile eggs was independent of the age of the females ($P>0.05$). Therefore, it can be concluded that every individual within 8 days after emergence has an equal ability to mate. The differences shown in Table 3 probably are caused by the abnormal egg deposition of older virgin females, since it is probable that their urge to lay eggs increases with age in spite of their virginity.

Harcourt (1957) found that mating began at dusk on the day of emergence. Sakanoshita and Yanagita (1972) also found that female DBM copulated at night on the day of emergence. Sarnthoy et al. (1989) reported that the preovipositional period was short and less than 1 day

Table 2. Number of DBM females that laid eggs irrespective of their fertility. Forty pairs were tested in each of the combinations.

Female age (days)	Male age (days) 0	2	4	6	8	Total	%
0	24	29	28	22	27	130	65.0
2	31	29	35	24	30	149	74.5
4	39	37	29	36	29	170	85.0
6	32	35	40	35	35	177	88.5
8	37	38	37	38	37	187	93.5
Total	163	168	169	155	158	813	
%	81.5	84.0	84.5	77.5	79.0	81.3	

Table 3. Proportion of DBM females that laid fertile eggs.

Female age (days)	Male age (days) 0	2	4	6	8	Mean
0	.667	.862	.893	.727	.852	.800
2	.677	.690	.657	.292	.667	.597
4	.462	.852	.655	556	.724	.650
6	.250	.514	.600	.743	.571	.536
8	.459	.710	.649	.553	.459	.566
Mean	.503	.726	.691	.574	.655	.630

when they were reared at 23.3°C. These studies clearly indicate that a female DBM mates at an early adult stage. The early mating and oviposition appear to contribute to their high reproductive rate. Both sexes maintain the physiological conditions possible to mate for a long period even if they fail in early mating.

Ohira (1979) studied the relationship between mating ability and degree of the development of ovarian eggs, and estimated the proportions of the individuals having mating ability. According to his estimation, 54% of 1-day-old, 40% of 2-day-old, 68% of 3-day-old and 98% of 4-day-old individuals mated. These figures show that the mating abilities of virgin females change with age, and differ from the results of this study. This disagreement probably stems from the simple assumption on mating ability which is determined by a single factor: degree of development of ovarian eggs.

Effect of Body Size on Mating Success

Of the 855 pairs tested, 100 pairs laid no eggs. Those pairs that failed to lay eggs were more frequently small females, i.e. 27.3% in the case of the smallest females, 5.1% in the case of the largest females, and about 10% in the case of females of intermediate size. This suggests that large females easily lay eggs.

The proportions of females laying fertile eggs to total number of females laying eggs irrespective of their fertility is shown in Table 4. Although they varied from 0.50 to 1.00, they were independent of the size of both sexes ($P > 0.05$). This suggests that the females have an equal chance to mate regardless of their body size.

The relationship between the degree of the size difference of paired insects and their mating success is shown in Table 5. When the difference was 2 mg or less, most of the females succeeded in mating and laid fertile eggs. When the difference was 3 mg or more, the probability of mating success was reduced to 63.5%. This suggests that large intersexual differences in body size has some ill effects on mating behavior of the pair. However, this factor seems unable to work

Table 4. Proportion of DBM females that laid fertile eggs.

Size class female (mg)	Size class male (mg)				Mean
	3.0-3.9	4.0-4.9	5.0-5.9	6.0-6.9	
3.0-3.9	.895	.808	(1.00)[a]	(.667)	.843
4.0-4.9	.817	.877	.788	(1.00)	.874
5.0-5.9	.845	.750	.847	.706	.787
6.0-6.9	(.500)	.800	.843	.966	.777
7.0-7.9	(.667)	.677	.857	(.824)	.756
Mean	.747	.782	.867	.833	.807

[a]Values in parentheses indicate data from small samples less than 20.

Table 5. Effects of size difference between both sexes in DBM on mating success.

Difference between female and male (mg)	No. of pairs tested	No. of pairs laying eggs		A/(A+B) × 100
		Fertile (A)	Unfertile (B)	
0	211	170	23	88.1 (83.4-92.7)[a]
1	391	273	69	79.8 (75.4-84.2)
2	192	142	26	84.5 (79.2-89.9)
3 or more	61	33	19	63.5 (50.0-76.9)

[a]Ranges for 95% confidence limit.

in natural populations, as the mean difference in weight between both sexes was less than 2 mg throughout the year (Uematsu et al., unpublished data).

In the present study, proportions of the females laying fertile eggs were considerably lower in contrast to those of previous studies (Uematsu, unpublished data). The reason for this seems to be in the methods used. If most of the females began to lay eggs the night after the day of mating (Sakanoshita and Yanagita 1972), females in the present study would not have had enough time for mating and oviposition. Because a period of 2 days was fixed for the experiment, if the females copulated during the night of the second day, they would not have had a chance to lay fertile eggs. They might then be recorded as ones not laying eggs, or ones laying unfertilized eggs if oviposition occurred at the first night. This is probably the reason for a small number of females laying fertile eggs in the present study.

Unfortunately, the reason why some females failed to lay fertile eggs was not determined in this study. Maa et al. (1985) reported that humidity is an important factor in inducing male response to the synthetic sex pheromone. Other physical factors, such as size of container, may also influence the process. Investigation of these factors needs to be done.

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4

Hibernation and Migration of Diamondback Moth in Northern Japan

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Abstract

It was assumed that one of the most important factors preventing the hibernation of the diamondback moth, *Plutella xylostella* (L.), in northern Japan is the low temperatures, 0°C or below, and snow cover during the winter. To test this hypothesis, individuals of each developmental stage of diamondback moth were reared at 15°C in the laboratory and kept at 0°C for varying periods of time and returned to 15°C. The mortality of chilled insects increased with the duration of chilling. All larvae and pupae died after chilling for more than 60 days. Although 7.5 – 10% of adults survived after chilling, none of their eggs could hatch normally. These results indicate that hibernation of diamondback moth in fields is impossible in areas where continuous snow cover lasts longer than 60 days. This condition exists in Hokkaido and a large part of Tohoku and Hokuriku districts of Honshu. Since Morioka city is located in the northern part of Honshu Island (39°42'N), diamondback moth hibernation in this area is impossible. A large number of adults, however, were caught by pheromone traps from April to November in Morioka. The first oviposition was observed in the latter part of May and adults which grew from these eggs began to emerge in mid June. Therefore, diamondback moth adults trapped from April to May could not be considered to have hibernated and emerged in Morioka City. This strongly suggests that these moths were migrants from the southern part of Japan.

Introduction

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), is a worldwide pest of crucifers, and it is found in most parts of Japan, from Okinawa to Hokkaido. The northern limit of its hibernation area in Japan was reported as the plains of Miyagi Prefecture (38-39°N) in Honshu (Maeda and Takano 1984). It was therefore assumed that the hibernation of DBM would be difficult in the northern part of Honshu and Hokkaido where the temperatures during the winter are lower than in Miyagi Prefecture (Honda and Miyahara 1987; Kimura et al. 1987).

The super cooling points for DBM larvae and pupae were reported as -14.3°C and -19.2°C, respectively (Hayakawa et al. 1988). In the northern part of Honshu Island, however, most of the agricultural fields are covered with snow during the winter, and it is unusual for the ground temperature to go below -15°C. Therefore, it is not likely that DBM larvae and pupae are killed by freezing during the winter in this area. Honda and Miyahara (1987) felt that one of the most important factors preventing the hibernation of DBM is the continuous cold, below 0°C, caused by the snow cover. They believed that DBM is not able to hibernate in areas where continuous snow cover exceeds 2 months.

Morioka City is located in Iwate Prefecture, in the northern part of Honshu Island where hibernation of DBM is supposed to be impossible. Nevertheless, a large number of DBM are caught by pheromone traps from spring to autumn, and many larvae and pupae are found on

various cruciferous plants. Since it is unlikely these DBM hibernated in the area, they must have been produced by adults which migrated from the southern part of Japan.

My objectives in this paper are the following: First, to examine the effects of continuous coldness (0°C) on the survival of different stages of DBM. Second, to determine the nonhibernating area of DBM in Japan. Third, to investigate DBM survival on overwintering cruciferous plants, the seasonal changes of DBM densities caught by the pheromone traps or observed on cabbage (*Brassica oleracea* var. *capitata*) in Morioka City, and the spring migration of DBM in northern Japan.

Effects of Continuous Coldness on Survival

DBM larvae and pupae were collected from cabbage fields and reared on cabbage leaves at 15°C, 12 hours light and 12 hours dark, for two or three generations in the laboratory. Eggs, second or third instar larvae, fourth instar larvae, pupae and adults of reared populations were kept at 0°C for different periods of time. The insects were returned to 15°C conditions after the chilling, and the number of survivors determined. The rearing temperature was first lowered to 10°C for 2 days. The temperature was then lowered to 5°C for another 2 days. And finally, the temperature was lowered to 0°C. This process was reversed when the insects were restored to 15°C conditions. The number of individuals used for each experiment was 50 for eggs to pupae and 20 for adults, and all experiments were repeated three times.

Eggs that could hatch after the chilling were regarded as survivors. Larvae and pupae were reared at 15°C after the chilling, and individuals that could successfully grow to adults were regarded as survivors. Some larvae and pupae which were alive just after the chilling failed to survive. Adults that could walk after the chilling were counted as survivors. The adult male and female survivors were put together in petri dishes with small cabbage leaves and a piece of cotton soaked in 0.1% sucrose solution, and the number of oviposited eggs and their hatchability were examined.

The mortality of chilled insects increased with the duration of chilling (Fig. 1). All eggs died when they were chilled over 50 days. All second to fourth instar larvae died when they were chilled over 40 days. Survival rates of pupae were higher than those of eggs and larvae,

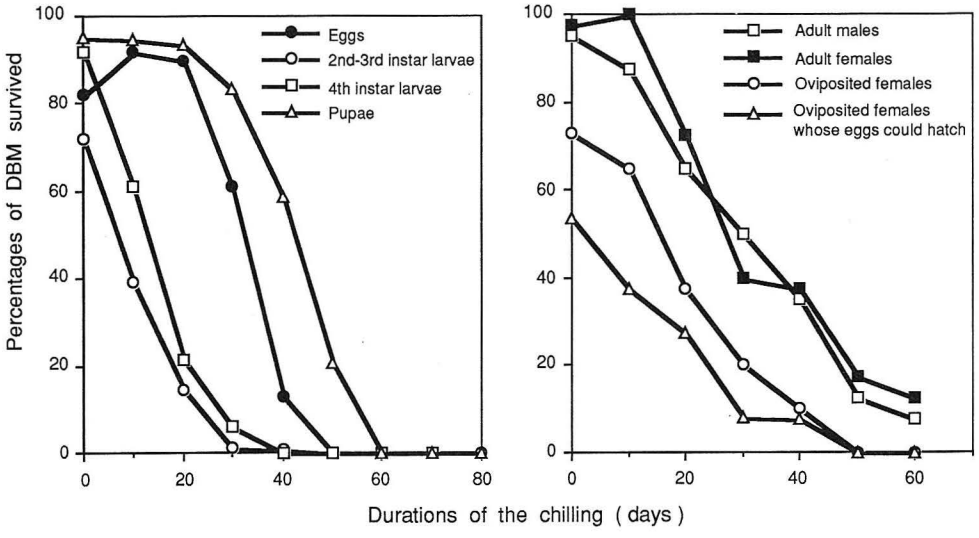

Fig. 1. Relationship between the duration of chilling and survival rates of the different developmental stages of DBM (Honda, unpublished data).

but all of them died when they were chilled over 60 days. Survival rates of adults were the highest of all developmental stages, and 7.5% of males and 10.0% of females were alive after they were chilled for 60 days.

Percentages of females which could oviposit after chilling were low, and the number of oviposited females became zero when they were chilled over 50 days. The number of females whose eggs could hatch was lower than the number of oviposited ones, and it also became zero when they were chilled over 50 days. Therefore, it is considered that all immature stages and adults of DBM cannot survive or oviposit when they are chilled at 0°C for more than 60 days.

Nonhibernating Area of DBM in Japan

The results suggest that DBM cannot survive or reproduce in places where the temperatures are 0°C, or below, continuously for 60 days. The temperature of cabbage leaves under the snow was nearly 0°C and it was constant in spite of the fluctuations of the temperature measured at 1 m above the ground (Fig. 2). In northern Japan, snowfall is common in winter and the ground is usually covered with snow for long periods. The duration of continuous snow cover is therefore considered a useful index to estimate the nonhibernating areas of DBM in Japan.

The area where the duration of continuous snow cover is longer than 60 days is shown in Fig. 3, including Hokkaido and a large part of Tohoku and Hokuriku districts of Honshu. The hibernation of DBM is supposed to be impossible in these areas, making the northern limit of DBM hibernation the plain area of Miyagi Prefecture.

There are some places where the duration of continuous snow cover is shorter than 60 days, for example in the seaside areas of the northern part of Honshu. Small populations of DBM may hibernate in these areas. For example, DBM hibernation was reported in the seaside area of Yamagata Prefecture (38°54'N) (Ishigaki et al. 1990).

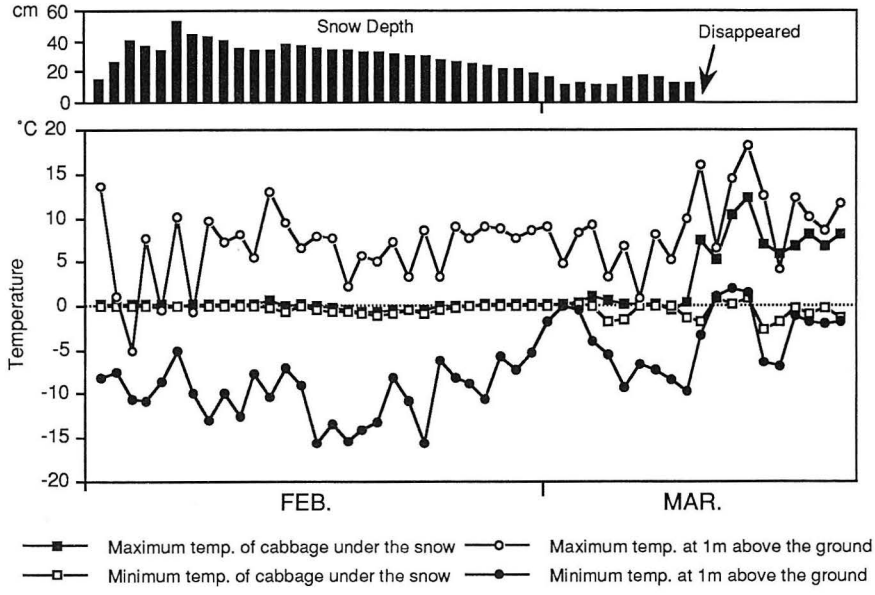

Fig. 2. Daily maximum and minimum temperatures of a cabbage plant under the snow, compared with temperatures measured at 1 m above the ground. Temperatures were measured with copper-constantan thermocouples which were covered with white filter paper and located both in the center of the cabbage and above the ground (Honda, unpublished data).

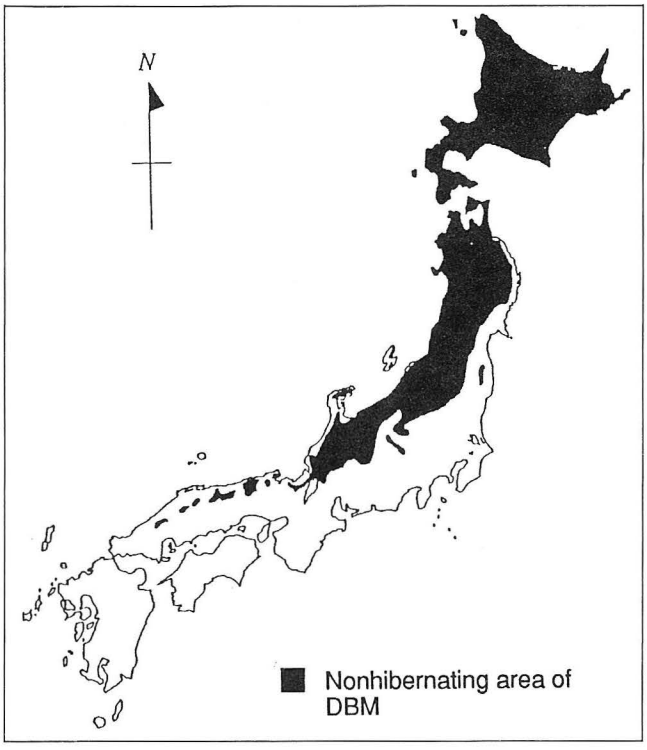

Fig. 3.
Nonhibernating area of DBM in Japan suggested by the results of chilling experiments. The dark area is the area where the duration of continuous snow cover is longer than 60 days.

Field Surveys of DBM Hibernation in Morioka City

The average duration of continuous snow cover is 79.8 days in Morioka City, making DBM hibernation impossible. To test this theory, surveys were carried out in 1985-86 on two fields (each 0.1 ha) of rape (*Brassica napus* subsp. *oleifera*) and on one 0.01 ha field of Chinese cabbage (*Brassica campestris pekinensis*) where 200 plants were cultivated. The fields are located in the Tohoku National Agricultural Experiment Station, Morioka City. These fields were sown or planted in September 1985 and cultivated until April 1986. Fifteen to twenty plants were sampled from the Chinese cabbage field in November, March and April and they were dissected to find DBM. Densities of DBM in 0.25 m^2 quadrats (50 × 50 cm) were checked on two rape fields in October, November, March and April, where 10 quadrats were randomly placed per field.

Live third or fourth instar larvae and pupae were found at densities of 5.3 and 3.3 individuals/10 plants, respectively, from the Chinese cabbage sampled on 22 November. Living individuals, however, could not be found from the plants sampled on 15-20 March, when these plants were under the snow, and on 10 April 1986. Live third or fourth instar larvae and pupae were found also in the rape in October and November at densities of 12.0 and 5.0 individuals/m^2 on 21 October, and 7.2 and 4.6 individuals/m^2 on 20 November, in two fields, respectively. In the spring, however, live DBM individuals could not be found in the same fields on 28 March, 18 and 28 April. These results support the contention that hibernation of DBM is impossible in Morioka City because of the duration of snow cover.

Seasonal Changes of DBM Density Around Morioka City

Seasonal changes in the number of DBM adults caught by the pheromone traps in Morioka are shown in Fig. 4. These traps were located in a white clover (*Trifolium repens*) field (A)

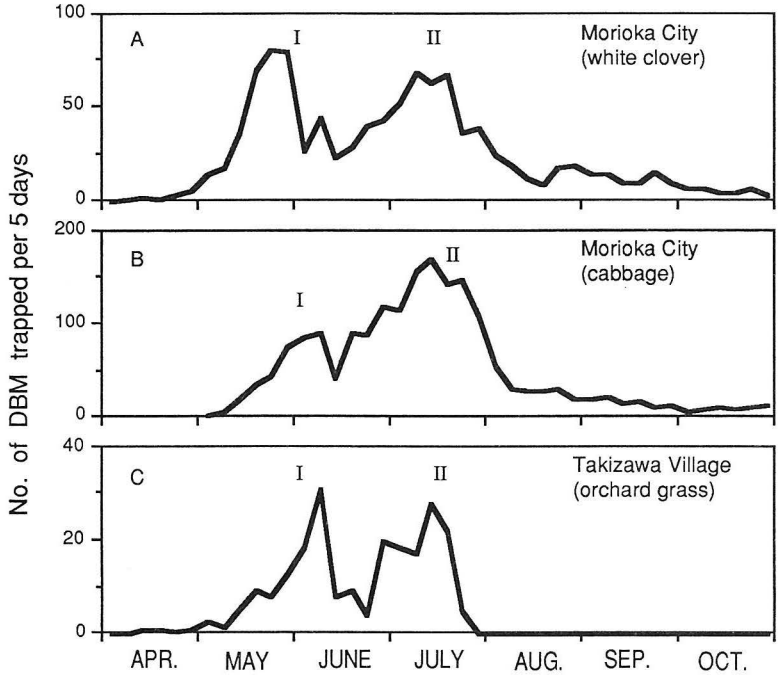

Fig. 4. Seasonal changes in the number of DBM adults caught by the pheromone traps in Morioka City and Takizawa Village. Values of the number of DBM adults are the average of 5 years (1983-87) in A, 4 years (1984-87) in B and 2 years (1986-87) in C. (The data were collected by the Laboratory of Entomology in Tohoku National Agricultural Experiment Station.)

and a cabbage field (B) in the Tohoku National Agricultural Experiment Station. The values in vertical axis are the totals of DBM adults caught in 5 days and these are the means of 4 or 5 years. The pheromone trap was a water pan-type (36 cm diam and 12 cm deep). A lure of DBM pheromone was a rubber septum on which Z-11-hexadecenal, Z-11-hexadecenyl acetate and Z-11-hexadecenol were coated in the ratio of 5:5:0.1 (0.1 mg/septum) (Koshihara et al. 1978), which was made by Takeda Chemical Industries, Ltd. The rubber septum was renewed every month. In the cabbage field (about 0.01 ha), 200 cabbage plants were continuously cultivated from May to October.

DBM adults were caught by the traps from April to November in Morioka each year. The number of adults increased during May-July and there were two peaks in the number of adults in May (I) and July (II) as shown in Fig. 4. The number of DBM adults at these two peaks was about equal to those in the white clover field. In the cabbage field, however, the number of adults at the peak in July (II) was much larger than that for May (I). This increase of DBM in the cabbage field in July can be explained by the reproduction of this insect on cabbage plants. Similar seasonal changes in the number of DBM adults were also observed in Takizawa village, 10 km west of Morioka, where the trap was placed in orchard grass (*Dactylis glomerata*). The number of DBM adults increased in May (I) and July (II) (Fig. 4-C).

The seasonal changes in the densities of immature stages of DBM and newly emerged adults at the cabbage field are compared with the number of adults caught by the pheromone trap in Fig. 5. This cabbage field was planted at the beginning of May 1986. Oviposition of DBM started in the latter part of May and the density of eggs increased at the beginning of June. The density of larvae increased in mid June and that of pupae increased in the latter part of June. Emergence

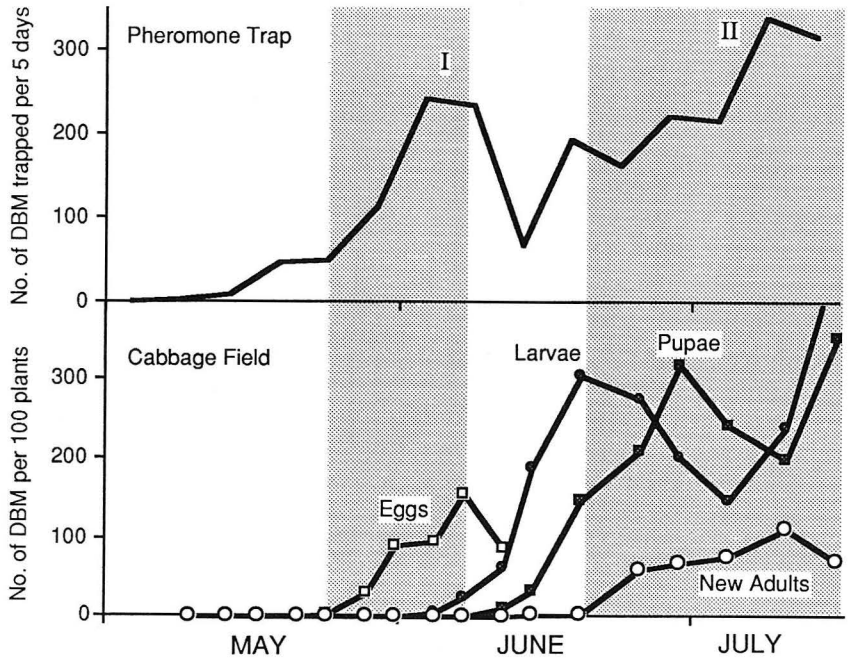

Fig. 5. Seasonal changes in DBM densities in a cabbage field, compared with the number of adults caught by the pheromone trap. Shaded parts show that the increase of eggs or new adults on the cabbage plants occurred at the same time as the increase of adults caught by the trap. (The data were collected by the Laboratory of Entomology in the Tohoku Agricultural Experiment Station.)

of new adults, which was estimated by the occurrence of empty pupal cases, started in mid June and the number increased at the end of June and early July.

The beginning of oviposition and the increase in eggs in the cabbage field occurred at the same time as the first increase of adults (I) caught by the trap. The increase of newly emerged adults in the cabbage field also occurred at the same time as the second increase of adults (II) caught by the trap. These results suggest that DBM adults that appeared in May oviposited in the cabbage field, and the emergence of the next generation of adults that grew on the cabbage plants caused the second increase in adults caught by the trap.

Discussion

Investigations of the seasonal changes of DBM densities showed that adults that emerged from the end of June in Morioka were derived from offspring of the adults that appeared in May. Therefore, DBM adults caught in July can be regarded as the newly emerged adults that grew on various cruciferous crops or wild plants around Morioka. On the other hand, according to the results of our 1985-86 field surveys, DBM adults that appear in the spring cannot be regarded as ones that hibernated or emerged there. It is likely, therefore, that those adults found in April-May in Morioka are migrants from the southern part of Japan.

The notable increase of DBM adults in May, which was considered to be caused by migrating individuals, was observed in both Morioka and Takizawa village. This suggests that there is a large-scale DBM migration in the spring covering a wide area.

In Europe, it has been established that DBM migrate for long distances, often 1000 km or more, with a storm caused by low atmospheric pressure (French and White 1960; Shaw 1962; Lokki et al. 1978). Harcourt (1957) and Smith and Sears (1982) showed that DBM cannot hibernate in Ontario, Canada, and a spring migration from southern United States likely occurs.

In Japan, the following examples show the extent of DBM migration: adults were caught at the top of a 1200 m mountain (Yamashita 1964); adults were caught on meteorological observation ships at sea (Asahina and Turuoka 1970); there was a sudden increase in adults following a typhoon (Miyahara 1986).

However, there have been few studies on the seasonal migration of DBM in Japan. The spring migration of DBM probably occurs not only around Morioka but also in the whole area of northern Japan. More intensive investigation of DBM seasonal migrations in this area are necessary, however.

The mechanisms of DBM migration and the source areas of migrants are not yet clear. One possible mechanism which can carry DBM migrants is a strong south wind caused by low atmospheric pressure systems. In the spring and early summer, there are many low pressure systems from northeastern China and the Korean peninsula to northern Japan. DBM adults could migrate with the strong south winds caused by these low pressure systems from the southern part of Japan, where DBM density increases in the spring.

In addition to the strong south wind, other factors probably contribute to DBM migration: the density of source population in the area of hibernation; the meteorological conditions when adults take off; the physiological condition of migrants; and the temperature of the upper air in which they migrate. The relationships between these factors and DBM migration need further study.

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5

Seasonal Variation in Populations of the Principal Insects Causing Contamination in Processing Broccoli and Cauliflower in Central Mexico

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Abstract

The seasonal variation in populations of insects causing product contamination in broccoli and cauliflower was measured in the central plateau of Mexico using black-light traps, pheromone traps, and plant surveys. The major species that are found in heads of broccoli and cauliflower are diamondback moth *Plutella xylostella* (L.), and cabbage looper *Trichoplusia ni* (Hübner). Pheromone traps collected both species all year, whereas the black-light trap only collected diamondback moth during the spring. In pheromone traps and plant surveys, diamondback moth was found in significant numbers from April through October. Populations of cabbage looper showed extended peaks in spring and autumn when measured by pheromone traps. Plant surveys showed cabbage looper larvae to be highest from May through September. *Artogeia rapae* and *Leptophobia aripa* populations increased beginning in September. They laid more eggs and produced more larvae on broccoli than on cauliflower.

Introduction

For many agronomic and economic reasons the central plateau of Mexico has become a major center of broccoli and cauliflower production to supply the increasing demand for these products from the United States. The plateau area is located at a latitude of approximately 20°N and an altitude of 1700-2000 m. The climate is temperate and relatively free of seasonal variation.

Insect control was not considered a major problem until 1986-87. At that time, control of the diamondback moth (DBM) *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) became the major preoccupation of the farmers and processors in the region. To effectively implement Integrated Pest Management (IPM) programs, it is first necessary to monitor the seasonal variations in pest populations. In this paper, we are reporting the activity of adult populations of the principal insect pests as monitored by black-light and pheromone traps and the corresponding egg, larval and pupal contaminants found in field surveys. Parasitism of the two major species is reported along with data on other important species encountered during the vegetative growth phase of the plant.

Procedure

The data reported here were collected on two separate ranches, El Copal near Irapuato and Villa Verde near Salamanca in the state of Guanajuato. The ranches are located about 30 km apart. El Copal is the research farm managed by the University of Guanjuato. On Villa Verde,

Gigante Verde maintains an experimental farm of 6 ha. Both farms are located in intensive agricultural areas where wheat, sorghum, garlic, sweet corn, broccoli, cauliflower and other vegetables are grown. Monthly plantings of broccoli (*Brassica oleracea* var. *italica* cv. Green Valliant) or cauliflower (*Brassica oleracea* var *botrytis* cv. Imperial 10-6) were made for the evaluation of pest populations. At Irapuato both crops were maintained to measure insect preferences. In Salamanca broccoli was planted in the winter and spring and cauliflower in the summer and fall (corresponding to normal cropping practices). These plantings were established using one application of malathion or endosulfan at transplanting. No other insecticides or fungicides were applied. Surveys were made in these plots for the principal insect pests. Normally 25 random plants were inspected for each sample. At Salamanca two dates of transplanting were surveyed each week, and the data presented are of the two oldest plantings.

Black-light trap data are recorded from a trap located at the El Copal ranch. The trap consists of two F15T8 General Electric fluorescent tubes and are mounted on a sheet metal frame with a container mounted below containing a dichlorvos strip for insect killing.

Pheromone data are from traps located on the Villa Verde ranch. Pheromones and Pherocon 1C sticky traps were purchased from the Trece Company, Salinas, California, USA. Two traps for each species were used for data collection. Pheromones for DBM were changed every 4 weeks, but on offsetting 2-week periods to reduce the problems of declining pheromone effectiveness. Trap height was adjusted to just above the height of the plants. Pheromones for cabbage looper (CL), *Trichoplusia ni* (Hübner) (Lepidoptera:Noctuidae), were changed every 2 weeks. New pheromones were placed in the traps on alternating weeks. Pherocon 1C cardboard sticky traps were used for DBM while large-mesh cone traps, usually used for *Heliothis*, are used for CL.

Results

Black-light trap — Irapuato

Black-light collections were started in April of 1989. Data from April 1989 to 15 October 1990 are presented in Fig. 1. The black-light collections of DBM show a very strong peak during May 1989. The black-light collections in 1990 showed a peak during the week of 25 March, and then substantial peaks in the last 2 weeks in May, similiar to the 1989 peaks. Essentially no DBM was collected in the black-light trap after 1 August in either year. More DBM males than females were collected in the black-light trap (357 to 165 in 1989, and 448 to 188 in 1990).

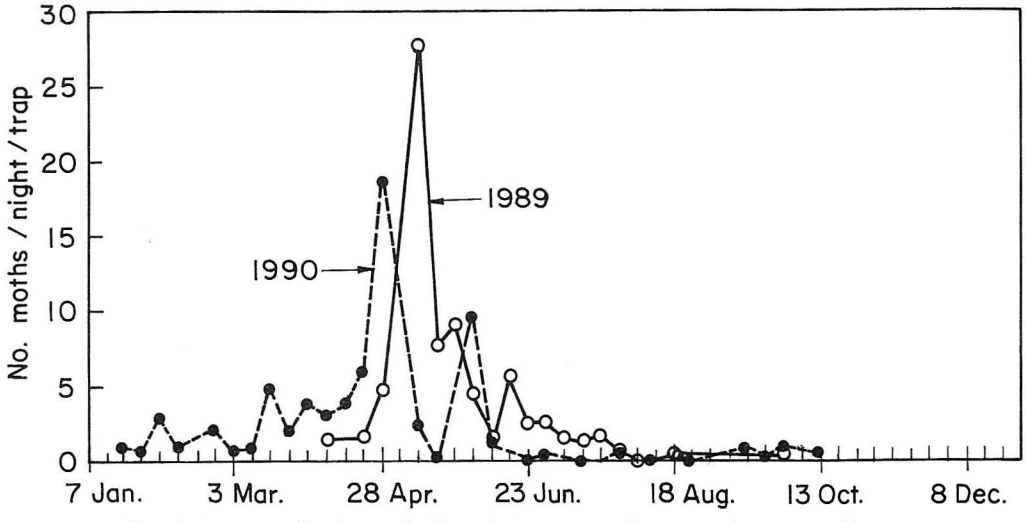

Fig. 1. Diamondback moth black-light trap collections, Irapuato, Mexico.

Of particular interest is the fact that this black light trap seldom collected CL, although CL eggs and larvae were found in the adjacent crucifer plantings. McCully (unpublished data) collected many CL moths in Maryland between 1965 and 1975 using a black-light trap. Chapman and Lienk (1981) reported collecting over 500 moths in July - November 1975 in western New York State. We have no explanation for the failure to collect CL adults.

The principal noctuid collected in the black-light trap was *Spodoptera frugiperda* (Hübner) (Lepidoptera: Noctuidae). From 1 April to 15 October 1989 and 1990, total collections of *S. frugiperda* were 3056 and 2870, respectively. This insect is seldom a pest or contaminant on broccoli or cauliflower.

Pheromone trap collections — Salamanca

Pheromone trap collections of DBM were started in October 1987. DBM moths have been collected every week, except one, since 1987. For discussion purposes, we will use an average of over 10 moths/night/trap as being a peak. Experience in production plantings would indicate that more than this number is likely to produce measurable field populations of DBM. The peaks are not definitive, lasting from 1 to 5 weeks as shown in Fig. 2. The data show six peaks in 1988, nine in 1989, and six in 1990. Data collected on other ranches showed peaks at times other than those shown here. This indicates that the DBM is responding to local conditions, most likely the crop stage. Therefore, to use pheromone traps to predict populations of DBM, the traps will have to be located on the grower's ranch.

CL trapping was started in January 1989 and the moths were found in the pheromone traps every week except one in 1989 (Fig. 3). In both years, two spring flights were recorded. In 1989, three peaks were noted in the fall. In both years, the lowest activity was reported in June.

Field collections

The major species recovered were DBM, CL, imported cabbage worm (ICW) *Artogeia rapae* (L.) (Lepidoptera:Pieridae), and *Leptophobia aripa* (Boisduval) (Lepidoptera:Pieridae). The strong preference of CL for laying eggs on cauliflower is shown in Table 1. ICW and especially *L. aripa* laid many more eggs on broccoli. Numbers of larvae and pupae recovered were different from the egg counts. CL and ICW were found in equal numbers on both broccoli and cauliflower. *L. aripa* eggs and larvae were found predominantly on broccoli (Table 2).

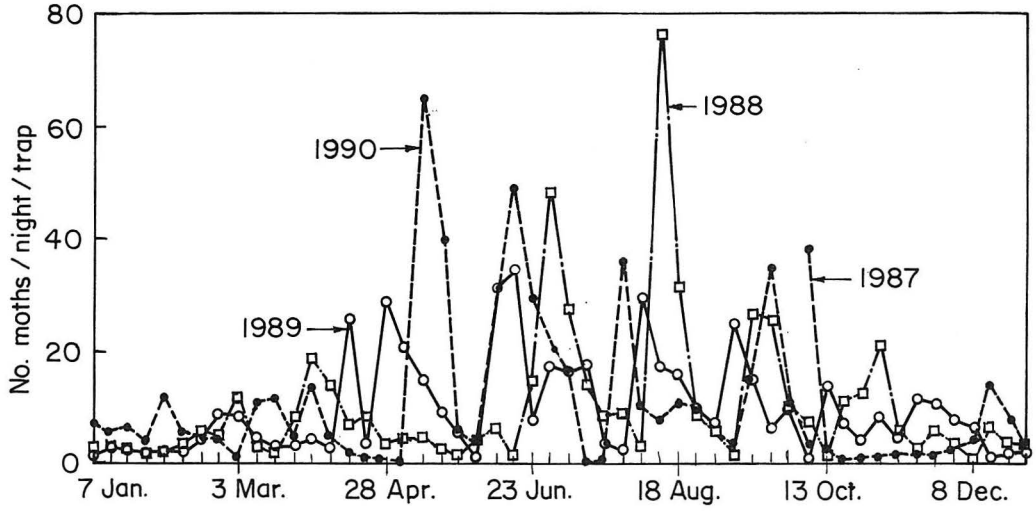

Fig. 2. Diamondback moth pheromone trap collections, Salamanca, Mexico.

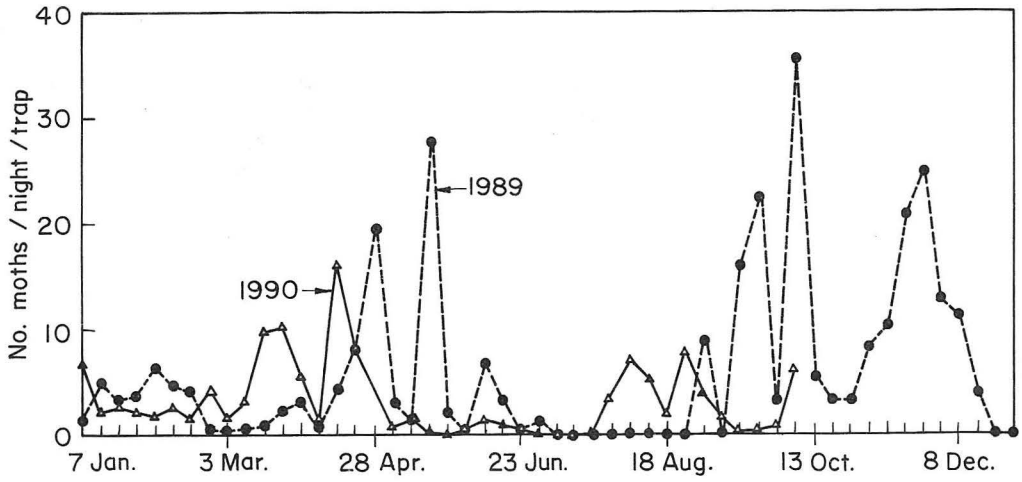

Fig. 3. *Trichoplusia ni* pheromone trapping results, Salamanca, Mexico.

Table 1. Influence of crop on the incidence of cruciferous pest eggs in 3 years, Irapuato, Mexico, 1990.

	Eggs/plant/sample					
	Broccoli			Cauliflower		
	1988	1989	1990	1988	1989	1990
T. ni	0.06	0.16	0.23	0.11	2.80	0.44
A. rapae	1.04	0.15	0.20	0.21	0.25	0.17
L. aripa	1.16	0.80	0.12	0.02	1.14	0.14

Table 2. Influence of crop on the incidence of cruciferous pest larvae and pupae in 3 years (larvae and pupae/plant/sample). Irapuato, Gto., Mexico. 1990.

	Broccoli			Cauliflower		
	1988	1989	1990	1988	1989	1990
DBM	0.07	0.17	0.31	0.08	0.41	0.54
CL	0.15	0.27	0.49	0.19	0.26	0.39
ICW	0.34	0.17	0.14	0.02	0.29	0.10
L. aripa	0.77	0.55	0.26	0.26	0.25	0.31

Monthly averages of larvae and pupae per plant, from Irapuato, are shown in Fig. 4, demonstrating the seasonality of the four most common species. DBM was found in significant numbers from April through September. At Salamanca we recorded over one larva per plant 10 weeks in 1988, 6 weeks in 1989 and 9 weeks in 1990. The concentrations were in September and October 1988, March-May 1989, and April-June and September 1990. CL lagged a month, being found mostly during May-August. In Salamanca, CL larvae were above one per plant in August and September 1988, May-June 1989, and June-July 1990.

In Irapuato, ICW larva showed two peak populations, June-September and November-December. In Salamanca, high larval populations were recorded only in 1988.

Leptophobia aripa larvae were found during September-December. In Salamanca, *L. aripa* were found rarely. Production experience indicates *L. aripa* is very easy to control in commercial plantings and is not considered a major pest. We encountered larvae in the heads of broccoli for the first time in an insecticide trial in October 1990. This contrasts with reports by Havranek (1981) in San Cristobal, Venezuela, where *L. aripa* was reported as the major pest in cabbage. Salinas and Briceno (1981) also report that *L. aripa* is a major pest of all cruciferous crops above 1,000 m in many countries of South America.

The most common parasites encountered were *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae), and *Voria ruralis* (Fallen) (Diptera: Tachinidae). Parasitism percentages are shown in Table 3. DBM parasitism by *D. insulare* was about equal in both broccoli and cauliflower. In contrast, CL parasitism by *V. ruralis* was higher in broccoli in 1988 and 1990, and higher in cauliflower in 1989. Except in the case of broccoli in 1990, *D. insulare* parasitized a higher percentage of DBM than *Voria ruralis* of CL.

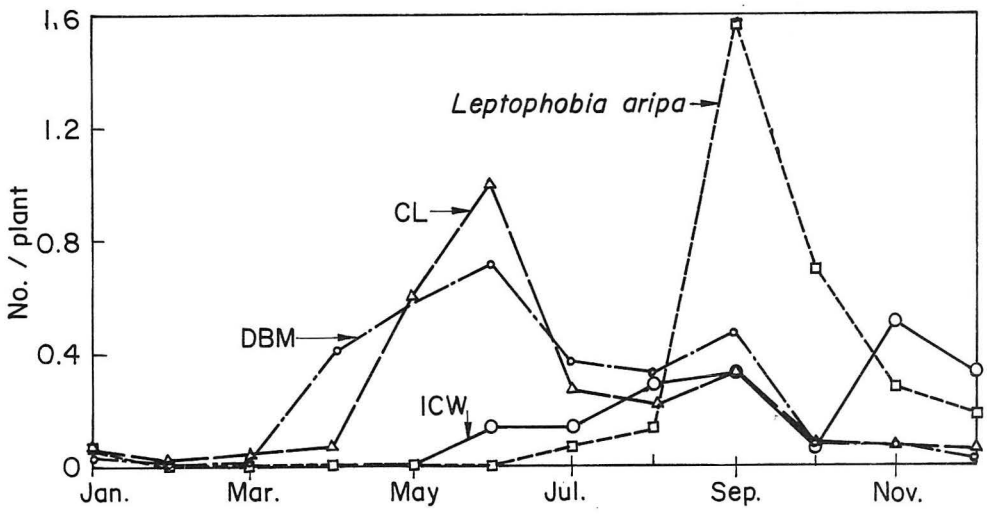

Fig. 4. Monthly averages of larvae and pupae/plant, Irapuato, Mexico (crops and years combined).

Table 3. Parasitism of DBM by *D. insulare* and CL by *V. ruralis*, Irapuato, Mexico, 1990.

	DBM			CL		
	1988	1989	1990	1988	1989	1990
Broccoli	62.5	36.0	30.2	14.7	6.7	38.8
Cauliflower	56.7	30.9	32.3	0.0	15.5	15.4

Various other insects have been collected from the heads of broccoli and cauliflower. To date, they have been serious only in isolated fields and for short periods of time. These include: *Copitarsia* sp. (Lepidoptera: Noctuidae), *Peridroma* sp. (Lepidoptera: Noctuidae), *S. frugiperda*, *S. ornithogalli*, and *S. exigua* (Lepidoptera: Noctuidae). The cabbage aphid *Brevicoryne rapae* (L.) (Homoptera: Aphidae) is a serious contaminant of broccoli heads. When the population of this aphid is high, larvae of *Allagrapta* sp. (Diptera: Syrphidae) are also found in the broccoli heads.

Lygus lineolaris (Palisot de Beauvois)(Hemiptera: Miridae) was found as an occasional pest of cauliflower. The nymphs are not found, but the adults feed on the cauliflower heads causing discoloration over large parts of the head, making these heads unusable.

Hernandez and Alvarado (unpublished data), of Campbells Soup Company in Villagran, Guanjuato, Mexico, reported on the aphid species attacking broccoli, their abundance, preference for leaf age, and parasitism. They report highest populations in winter plantings. This makes the cycle of pests complete. On a calendar year basis, the aphids appear first, followed by DBM in April, CL in May, and the Pieridae in August and September.

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6

Resistance and Susceptibility to Insect Pests in Glossy Genetic Lines of *Brassica oleracea* in Connecticut, USA

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Abstract

The glossy cauliflower PI 234599 has been used in breeding programs as a source of resistance to diamondback moth, *Plutella xylostella* (L.), and other lepidopterous larvae, but the relationship between insect resistance and glossiness has not been clear. One way to clarify this relationship is to find glossy lines carrying different genes for glossiness and test their resistance to insects. In the process, additional sources of resistance may be identified. Ten glossy lines of broccoli, cauliflower, kale, and collards with at least four different genes for glossiness (three recessive nonallelic genes and at least one dominant gene) were tested in the field under natural infestation for resistance. The insect species studied were imported cabbageworm (*Pieris rapae* (L.)), diamondback moth (*Plutella xylostella* (L.)), cabbage aphid (*Brevicoryne brassicae* (L.)), and flea beetles (mainly *Phyllotreta cruciferae* (Goeze), but also *Phyllotreta striolata* (F.)). All glossy lines were resistant to cabbage aphid and all except one was consistently resistant to imported cabbageworm. They were susceptible to flea beetles, although less susceptible in the fall than in spring. Their resistance to diamondback moth varied greatly among plantings, probably because of the low insect population in Connecticut. In order for these additional glossy lines to become useful sources of resistance to diamondback moth, they need to be tested and bred where this insect is a serious problem, instead of under low natural populations or artificial infestations in the northeastern USA.

Introduction

The first International Workshop on Diamondback Moth Management included two papers on resistance to diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera:Yponomeutidae), and to two other lepidopterous pests, the imported cabbageworm (ICW), *Pieris rapae* (L.), (Lepidoptera:Pieridae) and the cabbage looper (CL), *Trichoplusia ni* (Hübner) (Lepidoptera:Noctuidae), in the glossy cauliflower PI 234599 (Eckenrode et al. 1986, Dickson et al. 1986). It was clear from the breeding work of Dickson et al. (1986) that strong vertical resistance to these Lepidoptera could not be easily separated from the glossy character, but it was unclear whether resistance and glossiness were due to closely linked genes or whether glossiness itself was a factor in resistance.

In other studies, glossiness has been associated with lower populations of the cabbage aphid *Brevicoryne brassicae* (L.) (Thompson 1963; Way and Murdie 1965), and higher than normal populations of flea beetles, although no systematic measurements were made (Anstey and Moore 1954; Way and Murdie 1965; Dickson and Eckenrode 1980). In these papers it was also not clear whether the effects on insect populations were associated with glossiness in general or with other genes in a specific glossy genetic line.

Glossy plants appear dark green and shiny, compared to the bluish-white haze on the surface of plants with normal leaf wax. The glossy appearance is due to a change in the microscopic structure of wax on the leaf surface, with flat plates or sparsely distributed short rods or globules of wax, rather than the dense mat of vertical tubes characteristic of normal wax. These changes in wax structure are usually associated with a decrease in the quantity of wax per unit area, a change in chemical composition of the wax, or both (Baker 1974; Jeffree et al. 1976). At least eight different genes for glossiness have been found in *Brassica oleracea*, and substantial differences in the chemistry and wax morphology among these glossy lines have been described (Anstey and Moore 1954; North and Priestley 1962; Macey and Barber 1969, 1970; Denna 1970; Netting et al. 1972; Baker 1974; Jeffree et al. 1976).

The first step toward establishing the relationship between glossiness and resistance was to collect plants with several different genes for glossiness and test them for resistance. I tested them in the field under natural infestations of several insect pests. There would be three possible outcomes of this test with respect to any of the pest species to which PI 234599 was resistant: (1) only PI 234599 (called here Caul1) would be resistant and none of the other lines with other genes for glossiness would be; (2) all glossy lines would be resistant; or (3) some but not all of the lines with different genes for glossiness would be resistant. Any of these outcomes would help to narrow the search for the mechanism of resistance, and either of the last two would suggest additional glossy lines that could be used in breeding as sources of resistance.

Materials and Methods

Seed from the specific genetic lines described from 15 to 35 years ago was not available in most cases, but I did find seed of glossy lines from several sources in different crop morphotypes within *B. oleracea* (Table 1). I have at least four nonallelic genes for glossiness (three recessive and one dominant), and possibly more, since I have not yet determined allelism for the dominant

Table 1. Glossy lines tested for resistance to insects in 1988 and 1989.

	No. of plantings	Genetics of glossiness		Source[1]
		Dominance	Allelism	
Broccoli				
Broc3	7	dominant	?	Borchers
Broc4	6	dominant	?	Borchers
Broc5	4	recessive	not allelic	Sampson
Cauliflower				
Caul1	7	recessive	to KCR4, Gl.Vates	NERPIS (PI 234599)
Glossy Andes	4	recessive	not allelic	discovered in cv. Andes
Collards				
Green Glaze	7	partial dominant	?	Christianson
S.C. Glaze	7	partial dominant	?	Borchers
White's Gr. Glaze	7	partial dominant	?	Borchers
Kale				
KCR4	7	recessive	to Caul1,	Borchers
Gl. Vates	7	recessive	to Caul1 KCR4	Christianson

[1]Sources: Borchers = E. A. Borchers of the Hampton Roads Agricultural Experiment Station, Virginia Beach, VA, USA; Sampson = D. R. Sampson, Plant Research Centre, Ottawa, Ontario, Canada; NERPIS = Northeast Regional Plant Introduction Station, USDA-ARS Germplasm Resources Unit, NYSAES, Geneva, NY, USA; Christianson = Alf. Christianson Seed Company, Mount Vernon, WA, USA.

genes. As noted in Table 1, I have observed the insect populations under natural infestations on these genetic lines in 4-7 plantings for each line in 1988 and 1989. These observations were made at Lockwood Farm in Hamden, CT, or the Valley Laboratory in Windsor, CT. The glossy lines and normal standards were transplanted in early May for harvest in July-early August (spring planting), and in late July for harvest in late September-October (fall planting). All plantings were made in a nested randomized block design with three blocks and one row of each genetic line within each block. Observations were made on two plants from the interior of each row.

Figures 1-4 have been produced from selected data sets from 1988. The data from that year have been published in full (Stoner 1990). Reference will also be made to data from 1989. The glossy lines Broc5 and Glossy Andes were first tested in the field that year. These data have been analyzed, but are not yet published.

Results

ICW

The resistance of glossy lines to ICW is shown in Fig. 1. In the seven plantings in 1988 and 1989, all glossy lines of broccoli, cauliflower and collards and the glossy kale Glazed Vates consistently had lower numbers of ICW larvae than the lines with normal wax. The only exception to this pattern was one line with highly variable resistance, the glossy kale KCR4, which had numbers of ICW equal to those on some normal lines, including normal kales, in three out of seven plantings.

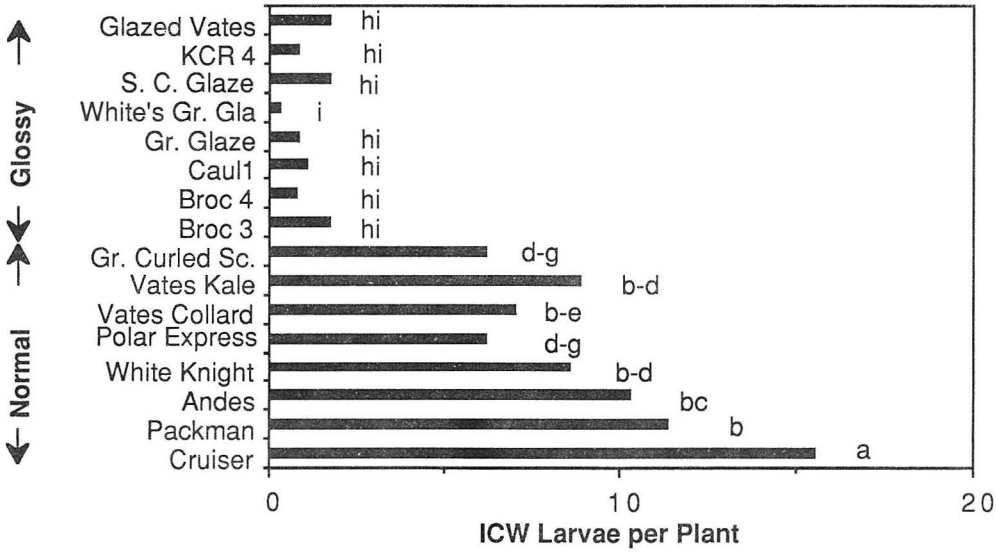

Fig. 1. ICW per plant in repeated sampling over the growing period. Bars followed by the same letter are not significantly different (P = 0.05; protected LSD test). Crop morphotypes of glossy lines are listed in Table 1. Crop morphotypes of normal lines are: Gr. Curled Sc. and Vates Kale--kale; Vates Collard--collard; Polar Express, White Knight and Andes--cauliflower; Packman and Cruiser--broccoli. Lockwood, fall 1988.

DBM

DBM, unlike ICW, occurred in substantial numbers only sporadically in my plots, and, in fact, did not occur in numbers large enough for comparison of different lines in any planting in 1989. As illustrated in Fig. 2, glossy lines as a group appear slightly more resistant than normal lines, but DBM numbers varied widely within both groups. The relative resistance of glossy lines also varied between plantings (Stoner 1990). Thus, the natural population level of DBM in Connecticut, as in New York (Eckenrode et al. 1986), is insufficient either in numbers or in uniformity of infestation for accurate screening for resistance.

Sanford Eigenbrode (University of California Riverside, pers. comm.) has tested many of the same glossy lines in the field in New York State using artificial infestation with DBM eggs and found that all the glossy genotypes studied except KCR4 had lower larval mining and survival than normal varieties of the same crop morphotype. He also found that levels of resistance were correlated with a low quantity of wax per unit leaf area and low density of crystalline structures per unit area under scanning electron microscopy.

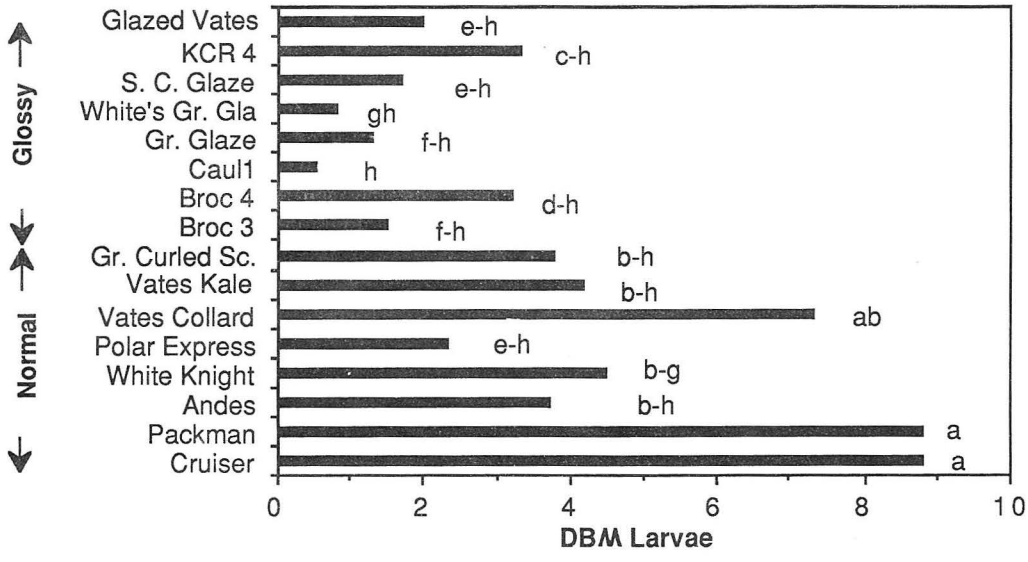

Fig. 2. DBM larvae per plant in repeated sampling over the growing period. Bars followed by the same letter are not significantly different (P = 0.05; protected LSD test). For identification of the lines, see Table 1 and caption of Fig . 1. Lockwood, fall 1988.

Brevicoryne brassicae

The data for *B. brassicae* are limited to three plantings, because these aphids occurred in substantial numbers in only one of the two locations, and not in all plantings at that location. But the pattern of low numbers of apterous *B. brassicae* on all glossy lines is consistent both within plantings (as illustrated in Fig. 3) and between plantings. When transformed back into antilogarithms, the mean numbers of aphids per plant were 100-313 for the more susceptible normal lines in this planting (Green Curled Scotch, Vates Kale, Packman and Cruiser), as compared to a maximum of 5.4 aphids/plant for the most susceptible glossy line KCR4.

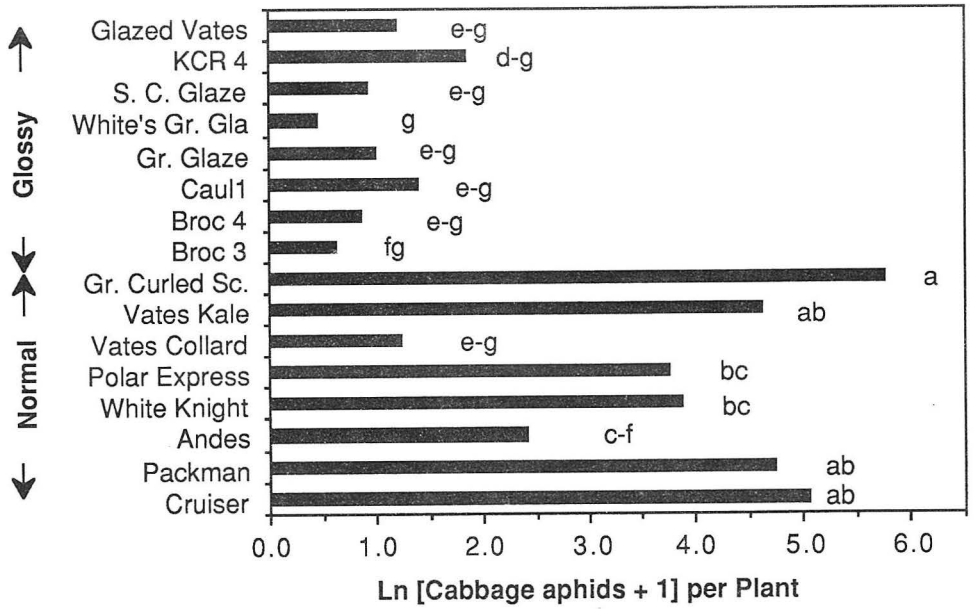

Fig. 3. Apterous cabbage aphids *Brevicoryne brassicae* per plant in repeated sampling over the growing period. Bars followed by the same letter are not significantly different ($P = 0.05$; protected LSD test). For identification of the lines, see Table 1 and caption of Fig. 1. Lookwood, fall 1988.

Phyllotreta cruciferae

As shown in Fig. 4, glossy plants had higher numbers of the flea beetle *P. cruciferae* than normal plants in some plantings. These data are from a spring planting in 1988; in the fall planting in 1988 at the same location, the trend of the data was reversed, and most normal lines had higher numbers than glossy lines, although the differences were not statistically significant. In 1989, glossy lines tended to have more *P. cruciferae* in both spring and fall, but the differences were not always statistically significant, particularly in the fall. Another species of flea beetle *Phyllotreta striolata* has a pattern similar to that of *P. cruciferae*.

Discussion

Most glossy lines were more resistant to ICW, *B. brassicae*, and DBM, and more susceptible to the flea beetles *P. cruciferae* and *P. striolata* than lines with normal leaf wax. However, the kale KCR4 was more variable in its resistance to ICW than the other glossy lines and has not shown any resistance to DBM. It was surprising that the level of resistance of KCR4 was so different from that of Caul1 because genetic tests indicated that these two lines (and also the kale Glazed Vates) have allelic genes for glossiness. This means that there are either modifying genes elsewhere in the genome that interact with glossiness genes (and possibly environmental factors) to affect resistance, or the genes for glossiness in these lines, although allelic, are not identical in their action, and these differences in action affect resistance. The difference in resistance between KCR4 and Caul1 and how it relates to differences in wax structure and chemistry in crosses and segregating populations between these two lines is currently under investigation.

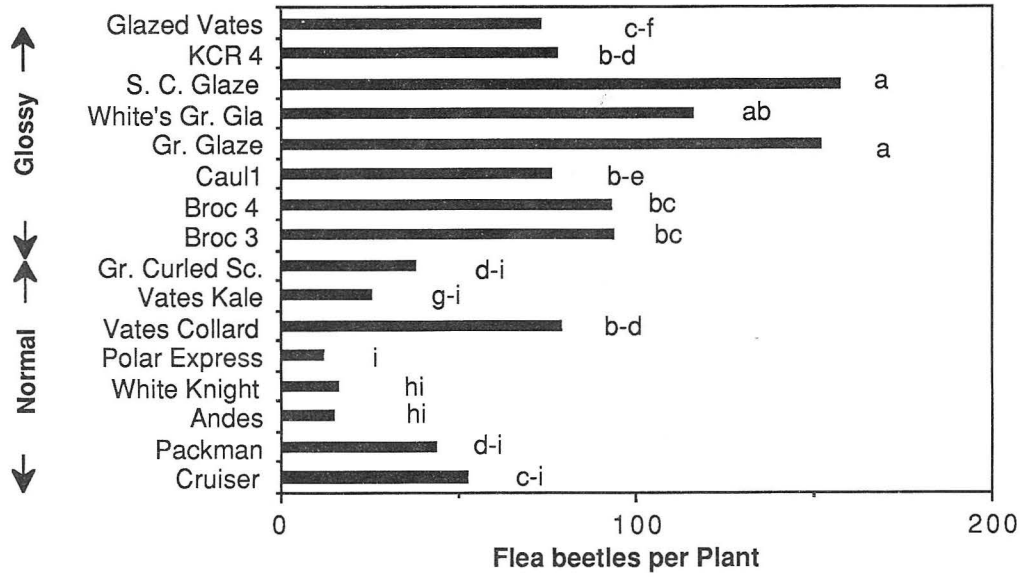

Fig. 4. Adult crucifer flea beetles *Phyllotreta cruciferae* per plant in repeated sampling over the growing period. Bars followed by the same letter are not significantly different (P = 0.05; protected LSD test). For identification of the lines, see Table 1 and caption of Fig. 1. Lookwood, spring 1988.

Aside from KCR4, the other glossy lines can be used as sources of resistance to ICW, *B. brassicae*, and DBM, and may present advantages to plant breeders, such as dominance, availability in other crop morphotypes, or avoidance of a single narrow genetic base, over the breeding material produced by Dickson derived from PI 234599 (or Caul1). In order for these lines to become useful sources of resistance to DBM, however, they need to be tested and bred under realistic conditions in regions of the world where DBM is a serious problem, instead of under low natural populations or artificial infestations in the northeastern USA.

Glossiness changes much more than just resistance to a few species of lepidopterous larvae. It increases resistance to the aphid *B. brassicae* (but there was no observed effect on the other common aphid species *Myzus persicae* (Sulzer)). As shown here, it may increase susceptibility to flea beetles. If, as Stork (1980) has proposed, glossiness affects the ability of all insects using adhesive setae to walk on the plant, glossiness may affect the movement of many predators and parasites (as observed by Way and Murdie (1965) for coccinellid and anthocorid predators) as well as pests. Water sticks to the surface of glossy leaves, instead of rolling off as it does on a normal waxy surface, a factor that could change the dynamics of plant and insect pathogens, the effectiveness of pesticides, and the effect of rain on many small insects. In my experience, glossy broccoli and cauliflower plants often produce heads later than normal plants, although this could relate to inbreeding or lack of local adaptation rather than the glossy trait itself.

In short, glossiness alters the entire ecology of the plant. In order to know if glossiness would fit into an integrated pest management system as a way of controlling DBM, all these changes in plant ecology must be observed under the conditions where the plant will ultimately be used.

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7

Resistance to Diamondback Moth in *Brassica*: Mechanisms and Potential for Resistant Cultivars

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Abstract

Resistance to diamondback moth, *Plutella xylostella* (L.), has been a major criterion for selection in the crucifer breeding program at the New York State Agricultural Experiment Station. Two types of resistance have been identified and studied. Larval survival on normal wax genotypes (with the typical whitish bloom of cultivated *Brassica*) is about 50% of survival on susceptibles. Polar extracts of the resistant plants reduce larval survival when added to an artificial diet, but the active compounds have not yet been identified. Larval survival on glossy wax genotypes (lacking the normal bloom) is reduced to as low as 1%, compared with standard cultivars. Glossy resistance is associated with reduced wax and reduced density of wax crystalline structures (crystallites) on leaf surfaces. Leaf wax load and crystallite density explain 69% of the variation in survival on a collection of 18 glossy and normal bloom genotypes of *Brassica oleracea*, based on regression analysis. Glossy leaf waxes apparently elicit nonacceptance behaviors in neonate larvae which result in their failure to successfully establish on these plants. Knowledge of resistance mechanisms will facilitate the development of *B. oleracea* cultivars resistant to diamondback moth.

Introduction

Genetic resistance to Lepidoptera is well documented in crucifers (Pimentel 1961; Radcliff and Chapman 1966; Brett and Sullivan 1974; Ellis et al. 1986; Shelton et al. 1988). However, the breeding program of M. H. Dickson at the New York State Agricultural Experiment Station has been the only long-term effort to develop crucifer breeding lines with economically significant levels of resistance to these insects. In collaboration with entomologists, Dickson has identified several sources of resistance to Lepidoptera, including the diamondback moth, *Plutella xylostella* (L.) (DBM) (Lepidoptera: Yponomeutidae) (Dickson and Eckenrode 1975, 1980). He has subsequently determined the inheritance of this resistance, and enhanced its expression in breeding lines (Dickson and Eckenrode 1980; Lin et al. 1983, 1984; Dickson et al. 1984, 1990). This work was the subject of two papers at the First International Workshop on Management of the Diamondback Moth (Dickson et al. 1986; Eckenrode et al. 1986).

In recent years progress in this breeding effort has been accelerated due to the development of higher resolution screening methods based on infesting plants with large, known numbers of insect eggs (Shelton and Dickson, unpublished data). The emergence of DBM as a major international pest has also led to more concerted efforts to select specifically for resistance to this insect.

Two types of resistance have been developed in Dickson's program. Descendants of PI 234599 having shiny (glossy) leaves (as compared with the whitish appearance of standard cultivated crucifers [normal bloom]), are highly resistant to DBM and other Lepidoptera. The glossy trait from PI 234599 is inherited as a simple recessive Mendelian gene (Dickson and

Eckenrode 1980). The other type of resistance occurs in breeding lines having normal bloom. Inheritance of resistance in normal bloom families is quantitative, and the degree of resistance is not as great as in glossy genotypes.

We determined that progress could be further accelerated if the specific mechanisms of each of these types of resistance were understood. This knowledge would lead to the development of more precise criteria for selecting and maintaining resistance. In the case of polygenically inherited resistance, a knowledge of mechanisms might lead to resolution of the resistance into several phenotypic components. Knowledge of specific mechanisms would also permit more efficient incorporation of resistant traits into genotypes with other desirable agronomic characteristics.

In this report we will discuss our investigations of the mechanisms of the two types of resistance in the New York program, and their implications for the development of cultivars resistant to DBM.

Resistance in Normal Bloom Genotypes

Our first concern was to establish the importance of antibiosis (Painter 1951) in normal bloom resistance. To study this, we measured the percentage of DBM larvae surviving to fourth instar in the field on three normal bloom resistant breeding lines of cabbage: NY 2506, NY 2503, and NY 2535. For comparison we also included a susceptible cabbage cultivar 'Round-Up,' and a highly resistant glossy genotype, NY 2518. We measured survival by infesting whole plants, or 4 cm diameter cages attached to the plants, with known numbers of DBM eggs. To determine the possible effects of the resistance on early instars, we also counted the number of mines on infested plants. Mines are only formed by feeding first instar insects (Salinas 1984).

Survival of DBM was reduced significantly on two of the three normal bloom genotypes, NY 2503 and NY 2535 (Table 1). Survival on NY 2506 was not different from the susceptible 'Round-Up.' As expected, survival on glossy NY 2518 was extremely low. These results corresponded well with a ranking of the five genotypes based on damage ratings in previous unpublished field trials ('Round-Up' > NY 2506 > NY 2503 > NY 2535 > NY 2518, from most to least damaged), except that NY 2506 was not significantly different from 'Round-Up' in our survival tests. We concluded that antibiosis was most important in producing the observed resistance in NY 2503 and NY 2535. The excellent correspondence between mine counts and survival (Table 1) also indicated that the resistance strongly affected the first instar larvae in all the lines.

Table 1. Survival of DBM larvae to fourth instar and mining by first instars on five cabbage genotypes.

Test	Normal bloom control 'Round-Up'	Glossy NY 2518	Normal bloom test lines NY 2506	NY 2503	NY 2535
			Percentage surviving		
Whole plant[a]	60.3 ± 11.6 a	0.2 ± 0.2 c	61.6 ± 3.6 a	42.9 ± 6.1 ab	30.2 ± 3.6 b
Leaf cage[b]	35.0 ± 7.8 a	0.8 ± 0.9 c	36.6 ± 9.9 a	13.3 ± 4.5 b	16.6 ± 6.4 b
			Number of mines[c]		
	46.6 ± 6.0 a	0.0 ± 0.0 c	59.8 ± 6.7 a	15.6 ± 4.7 b	13.0 ± 3.9 bc

Means (±SE) in each row with the same letter are not significantly different ($P < 0.05$; Fisher's Protected LSD). aANOVA ($F = 13.63$; df = 4, 37; $P = 0.0001$). bANOVA ($F = 6.13$; df = 4, 84, $P = 0.0002$). cANOVA ($F = 22.73$; df = 4, 32; $P = 0.0001$).

To determine the importance of plant chemistry in producing the resistance, we prepared plant extracts from each of the five genotypes and tested these against DBM in artificial diet. We prepared the extracts by boiling plant material in ethanol. We dried this extract and sequentially eluted the residue with hexane and water to obtain polar and nonpolar fractions. This method of extraction was intended to obtain a broad spectrum of potentially active compounds. The extracts would include glucosinolates, long associated with insect-plant interactions in Cruciferae (Verschaffelt 1910; Gupta and Thorsteinson 1960; Nayar and Thorsteinson 1963; David and Gardiner 1966; Hicks 1974; Städler 1978; Renwick and Radke 1983, 1987; Renwick et al. 1989; Reed et al. 1989; and others), and cardenolides, recently shown to be important in mediating oviposition for some crucifer specialist insects (Renwick et al. 1989). We added the fractions to diet at a concentration of 4 g leaf equivalents/g of diet. As in field experiments, we determined DBM survival to fourth instar on these diets.

Percentage survival of DBM is reported relative to survival on a control diet containing no plant extracts (Table 2). Survival was significantly less on diets with polar extracts of NY 2503 and NY 2535, than on the diet with extract of susceptible 'Round-Up.' Survival on diet with extract of NY 2506 was more similar to that on 'Round-Up.' This suggested to us that a polar extractable component of the leaves of the resistant genotypes was toxic to the larvae, accounting, at least in part, for the observed antibiosis on whole plants. Surprisingly, extract of the highly resistant glossy genotype, NY 2518, was not toxic to larvae. Thus glossy types do not depend on this type of extractable toxin for resistance. Nonpolar extracts of all genotypes were inactive and, in fact, enhanced survival of DBM.

Subsequent efforts to isolate the specific polar compounds responsible for activity in diet have been unsuccessful. This may be because the compounds are unstable, or because several compounds present in the whole extract must act together to substantially reduce larval survival.

In summary, we have established that resistance in normal bloom types is a type of antibiosis, as in the glossy types, and that this resistance affects mining by first instars (Eigenbrode et al. 1990). Resistance in the normal bloom types depends, in part, on the presence of extractable polar toxins, which have yet to be identified. Glossy plants do not depend on this type of toxin for the high levels of antibiosis exhibited in these plants.

Table 2. Percentage of *P. xylostella* larvae surviving on artificial diet treated with polar and nonpolar extracts of five cabbage genotypes.

	Normal bloom control 'Round-Up'	Glossy NY 2518	Normal bloom test lines NY 2506	NY 2503	NY 2535
Polar extract[a]	94.2 ± 4.2 a	93.1 ± 4.1 ab	84.6 ± 5.6 abc	76.3 ± 4.0 c	80.2 ± 4.4 bc
Nonpolar extract[b]	106.6 ± 4.5 a	106.3 ± 4.5 a	100.6 ± 5.1 a	117.3 ± 9.2 a	101.5 ± 5.7 a

Means (±SE) in the same row with the same letter are not significantly different ($P<0.05$; Fisher's Protected LSD). [a]TN = 388 (F = 3.32; df = 4, 377; P = 0.01) [b]N = 276 (F = 1.13; df = 4, 265; P = 0.344)

Resistance in Glossy Genotypes

Mutations which cause leaves to appear glossy are common in crucifers (Macey and Barber 1970; Netting et al. 1972; Baker 1974; Jeffree 1986). The recessive gene conferring this trait in PI 234599 is only one of these. Glossy genotypes of *Brassica oleracea* (L.), apparently unrelated to PI 234599, have also been reported to differ from normal bloom types in their susceptibility to insects (Anstey and Moore 1954; Thompson 1963; Way and Murdie 1965; Stoner 1990). It was already known that resistance in PI 234599 was a result of reduced DBM survival

(Lin et al. 1983), but the effect of genetically different glossy types on this insect was unknown. We therefore studied the effects of a genetically diverse collection of glossy *B. oleracea* genotypes on larval survival. Our goals were to look for new sources of resistance to DBM, and provide insight into the mechanisms of resistance based on glossy leaf.

We selected 18 genotypes for a test (Eigenbrode et al. 1991a). Seven of these were obtained from the collection of K. A. Stoner at the Connecticut Agricultural Experiment Station and had been assessed by her for field resistance to DBM (Stoner 1990, and chapter 6 in this volume). Ten were glossy genotypes from four horticultural types: two cabbage, two broccoli, two cauliflower, two collards, and two kales. Eight were normal bloom genotypes included for comparison: three cabbage, one broccoli, two cauliflower, one collard, and one kale. We infested the plants with DBM eggs and evaluated survival as above.

With one exception (KCR4 kale), survival on glossy genotypes was lower than on normal bloom types (Table 3). This difference in survival on the two leaf types was significant ($P = 0.0001$) among all the test lines and within crop types, except for kale. Inheritance studies demonstrated that at least three and probably more genes were represented in our test lines (Stoner 1990 and unpublished data; M. H. Dickson unpublished data). We concluded that, regardless of genetic source, the glossy leaf trait conferred some level of resistance to DBM, and that this was due to reduction in survival of the larvae.

Two questions remained: (1) What characteristics of glossy plants confer resistance to DBM? (2) How do these characteristics reduce survival of DBM larvae? These questions are interesting from a theoretical and applied point of view. Several experiments were conducted to answer them.

The most conspicuous feature of glossy plants is the unique appearance of the leaf surface, and we considered it possible that this was directly related to the resistance. Glossy mutants of *B. oleracea* previously characterized have less surface wax, fewer waxy crystalline structures on the leaf surface, wax structures of different morphology, and shifts in wax composition, relative to normal bloom types (Macey and Barber 1970; Netting et al. 1972; Baker 1974; Jeffree 1986). We measured leaf wax characteristics of our lines (Eigenbrode et al. 1991a) (Table 4). There was considerable variation in amount of wax (wax load) and density of crystalline structures (crystallite density) on the test lines. Glossy types had significantly reduced wax loads and crystallite densities compared to normal bloom types.

These differences suggest that specific wax characteristics may condition the resistance to DBM in glossy lines. Multiple regression analysis determined that wax load and crystallite density explained 69% of the variation in DBM survival, considering all 18 test lines, 31% of the variation in glossy lines only, and 78% of the variation in glossy lines if KCR4, the unusual susceptible glossy genotype, is excluded from the analysis (Table 5). We concluded that resistance depends to a large extent on these specific characteristics of the leaf waxes.

As an additional test we performed experiments in which the wax load and crystallite density on normal bloom genotypes was reduced by chemically disrupting the wax biosynthesis in the plants. This results in plants which closely resemble genetic glossy plants (Eigenbrode 1990). Plants so treated have wax loads and crystallite densities that are about one-half of those on treated plants. When these plants are infested in the field, survival of DBM is significantly reduced (average survival on controls 40.3% and on treated plants 23.1%).

To gain insight into the biology of the resistance, we conducted behavioral observations of the insects on resistant plants. Since the most vulnerable stage of the insect is the first instar, we developed methods to facilitate detailed observation of the behavior of this vulnerable stage of DBM on the plant surface (Eigenbrode and Shelton 1990; Eigenbrode et al. 1989). We found that neonate larvae move much more rapidly on resistant types than on susceptible types (Table 3) in our test lines. Based on regression analysis, movement rate of neonates explains 48% of the variation in survival on the test lines ($P = 0.0001$ for the model, and $P = 0.040$ for the b coefficient):

$$\text{Percentage surviving} = 27.71 - 31.58\ [\text{rate}].$$

Table 3. Survival of DBM at larvae and movement rates of first instars on *Brassica oleracea* genotypes.

	Crop type	Leaf type	Number of plants	Percentage of larvae surviving	Neonate movement rate (mm/min)
Genotype					
Andes	caul.	normal	23	29.2 ± 3.2 a	3.24 ± 0.19 efg
Imperial 10-6	caul.	normal	24	25.6 ± 3.9 ab	3.01 ± 0.18 fg
Round-Up	cabbage	normal	23	23.9 ± 2.9 ab	3.03 ± 0.25 fg
KCR4	kale	glossy	12	22.5 ± 5.2 bcd	3.96 ± 0.30 def
Vates Kale	kale	normal	18	22.3 ± 3.8 abc	4.02 ± 0.26 de
Market Prize	cabbage	normal	23	20.7 ± 3.9 bcd	2.82 ± 0.23 g
Vates Collard	collard	normal	23	19.3 ± 1.9 bcd	2.61 ± 0.34 g
Waltham	broccoli	normal	20	19.3 ± 2.6 bcd	4.03 ± 0.33 de
Ballhead	cabbage	normal	19	16.2 ± 1.7 cde	2.53 ± 0.24 g
Broc5	broccoli	glossy	22	14.8 ± 2.5 def	5.65 ± 0.37 c
Glazed Vates	kale	glossy	18	11.4 ± 3.0 efg	4.40 ± 0.31 d
Broc3	broccoli	glossy	23	8.9 ± 1.2 fg	4.03 ± 0.30 de
Glossy Andes	caul.	glossy	23	7.0 ± 1.5 gh	5.59 ± 0.17 c
PI 261597	collards	glossy	24	6.9 ± 1.1 g	3.22 ± 0.46 efg
PI 234599	caul.	glossy	17	5.6 ± 1.0 gh	8.44 ± 0.59 a
Greenglaze	collards	glossy	21	2.4 ± 0.3 hi	4.88 ± 0.53 cd
NY 8329	cabbage	glossy	18	2.0 ± 0.8 i	7.44 ± 0.48 b
NY 3891	cabbage	glossy	12	1.8 ± 0.4 i	7.58 ± 0.46 ab
Wax Type					
normal wax	all	normal	164	22.0 ± 1.1	3.16 ± 0.09
glossy wax	all	glossy	190	8.1 ± 0.7	5.56 ± 0.14*

Means (± SE) with same letter are not significantly different ($P<0.05$, LSD using transformed values). *Student's t $P = 0.0001$ (transformed data) **$P = 0.0001$.

Table 4. Physical characteristics of waxes on *Brassica oleracea* genotypes.

	Leaf type[a]	Wax $\mu g/cm^2$	Crystallites $0.001/mm^2$	Crystallite type
Genotype				
Andes	normal	57.5	1135.1	rods and filaments
Market Prize	normal	51.2	1044.5	rods and filaments
Round-Up	normal	52.8	922.5	rods and filaments
Ballhead	normal	58.9	831.3	rods and filaments
Waltham	normal	75.4	788.8	rods and filaments
Vates Collard	normal	60.7	628.3	rods and filaments
Vates Kale	normal	75.0	602.5	rods and filaments
Imperial 10-6	normal	58.9	587.4	rods and filaments
Broc3	glossy	20.4	203.8	short rods and polygons
Broc5	glossy	67.0	160.6	angular plates
PI 261597	glossy	59.4	112.5	irregular plates
KCR4	glossy	21.9	97.2	short rods and polygons
Glazed Vates	glossy	45.5	89.3	short rods and polygons
PI 234599	glossy	12.0	85.3	globules
Glossy Andes	glossy	13.9	65.8	polygons
NY 8329	glossy	11.0	31.4	globules
NY 3891	glossy	10.9	25.3	globules
Green Glaze	glossy	10.5	20.7	globules
Wax type				
normal wax	normal	61.3 ± 2.2	817.6±50.0	
glossy wax	glossy	27.5 ± 5.0*	88.8±13.6*	

[a]normal = normal wax, glossy = glossy wax *Student's *t*-test $P = 0.0001$

Table 5. ANOVA tables for linear regression of DBM percent survival on amount of wax ($\mu g/cm^2$) and wax crystallite density ($0.001/mm^2$).

Source	All genotypes				
	DF	MS	F	Prob>F	R^2
Regression	2	0.0463	17.12	0.0001	.69
Wax	1	0.0069	2.55	0.1310	
CD	1	0.0264	9.76	0.0070	
Error	15	0.0027			
Source	Glossy genotypes				
	DF	MS	F	Prob>F	R^2
Regression	2	0.0060	1.59	0.2692	.31
Wax	1	0.0012	0.33	0.5862	
CD	1	0.0046	1.24	0.3027	
Error	7	0.0037			
Source	Glossy genotypes (excluding KCR4)				
	DF	MS	F	Prob>F	R^2
Regression	2	0.0061	10.4	0.0112	.78
Wax	1	0.0030	5.07	0.0654	
CD	1	0.0026	4.34	0.0823	
Error	7	0.0020			

We proposed (Eigenbrode and Shelton 1990) that this higher movement rate on resistant types had biological significance. Specifically, we suggested that the higher movement rate indicated that neonates do not accept glossy plants as readily as normal bloom plants; they therefore spend more time walking and less time in gustatory exploration of the leaf surface, e. g. biting or feeding.

To establish a link between leaf waxes and larval behavior, we removed leaf waxes from resistant glossy NY 8329, and susceptible 'Round-Up,' with dichloromethane dips (30 sec) (Eigenbrode and Shelton 1990). After this treatment, neonate larvae moved slower on resistant types (2.7 vs 5.1 mm/min) and faster on susceptible types (2.6 vs 1.3 mm/min) so that the movement rates were statistically identical. If leaf wax crystalline structure was disrupted mechanically (by polishing with sterile cotton), neonate larvae moved no faster on NY 8329, but moved faster on 'Round-Up' (4.9 vs 2.9 mm/min) so that the rates were again statistically identical on treated plants of both genotypes. These studies indicated that leaf waxes, both quantity and crystalline structure, influenced larval behavior, as well as survival.

The 20-30% of variability in DBM survival not explained by wax load and crystallite density may be partly due to differences in wax crystallite morphology or chemistry. Wax crystallites on the most resistant lines, including PI 234599, which has an intermediate crystallite density, are in the form of globules. The wax crystallites of KCR4, 'Glazed Vates,' and Broc3 include short rods which resemble those found on normal bloom plants. These three genotypes are more susceptible than expected, based on their rank in crystallite density (Tables 1 and 3). The waxes also differ substantially in chemical composition, based on a comparison of their thin layer chromatographic patterns (Eigenbrode et al. 1991a, 1991b). Pure waxes from resistant and susceptible genotypes also elicit different behaviors from neonate larvae (Eigenbrode et al. 1991b). It seems probable that additional genetic variation for these characteristics of leaf waxes may be exploited to enhance resistance to DBM in crucifers.

In summary, all the above studies strongly suggest a causal link between wax characteristics and survival of larvae on the plants. Reduced survival is associated with larval nonacceptance of plants with glossy leaf wax characteristics. Incorporation of any gene conferring glossy wax traits should increase resistance to DBM. Our results also suggest that maximal expression of resistance will result from the lowest possible leaf wax loads and crystallite densities. It appears that these traits may be modified by minor genes, as well as the principle genes which condition the glossy trait. For example, during the enhancement of resistance to DBM, Dickson has also produced genotypes with wax loads and crystallite densities that are less than those of the original PI 234599 (Tables 3 and 4).

Potential for Development of Resistant Cultivars

Glossy Cultivars

The potential for the eventual release of glossy cabbage cultivars with resistance to DBM and other Lepidoptera is great. The extremely high levels of resistance obtained in some breeding lines (for example NY 2518, NY 8329, and NY 3891, Tables 1 and 3), and the simple inheritance of the glossy trait will favor this. In tests performed so far, glossy resistant lines, or hybrids derived from them, have performed well in advanced trials and production trials. Glossy lines from Dickson's program perform extremely well under pressure from high populations of insecticide-resistant DBM in Honduras (Dickson et al. 1990), where insect populations on glossy plants in Honduras were only about 2 % of those on susceptible controls. In production simulation trials in New York, glossy-leafed hybrid cabbages produced marketable crops without the aid of chemical sprays. In these same trials, the normal bloom susceptible cultivar 'Round-Up' required an average of 4.5 sprays with permethrin to produce a marketable crop (unpublished data).

Although the potential of glossy resistant lines is great, some difficulties have slowed cultivar development. First, although almost all glossy genotypes have some level of resistance

(Table 3), economically useful levels can only be maintained, and cultivars developed, by continued screening using insect infestations. Second, the dark green glossy foliage of resistant types is considered by many a horticultural, cosmetic defect. Third, a reduction in plant vigor is associated with the glossy trait. In F_2 populations segregating for the glossy trait derived from PI 234599, glossy plants are significantly smaller than normal bloom siblings 30 days after transplant (0.26 ± 0.01 kg vs 0.72 ± 0.4 kg; $P = 0.0001$). As a result, although glossy cabbage lines were released for use by breeders in 1984 (Dickson et al. 1984), DBM-resistant cultivars have not yet been produced.

We think these problems can be overcome. First, an alternative to the labor-intensive requirement to screen glossy plant populations using insects is now feasible. Based on our findings, screening for reduced waxes and reduced crystallite density could be used to maintain high levels of resistance in glossy lines. Second, the tremendous need for cultivars resistant to DBM may outweigh cosmetic considerations. This is especially true in areas where insecticide resistance in DBM is widespread. In addition, when stripped of the outer leaves for market, as is done in many tropical areas, glossy cabbages appear very similar to standard types. Third, although glossy plants are less vigorous which may affect yield, this too may be outweighed by the need for resistant cultivars, as reduced yield is preferable to none. For some markets, smaller cabbages are even desirable. It is also possible that the lack of vigor associated with gloss may be at least partially eliminated through additional breeding efforts.

Normal Bloom Cultivars

The normal bloom types only provide partial protection against DBM (Table 1). The incentive to develop partially resistant cultivars is lower than for highly resistant ones. Unless subsequent work can further enhance DBM resistance in the normal-bloom lines, it appears that the development of cultivars based on these lines will not occur soon. Nevertheless, in production trials, hybrids developed from normal bloom-resistant cultivars required only 3.0 sprays with permethrin to produce a marketable crop, as compared with 4.5 on susceptibles (unpublished data). This could translate into large savings in production costs in tropical areas where insecticide requirements are much greater than in New York.

Conclusions

DBM threatens crucifer production worldwide. The problem has been well documented (Talekar and Griggs 1986). The economic incentive for the development of DBM-resistant cultivars of cabbage and other *Brassica* crops by private seed companies is growing. As we report here, most of the biological problems with incorporating resistance to DBM have or can be overcome. We are hopeful that DBM-resistant *Brassica* cultivars will be released in the future.

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Cabbage Webworm on Crucifers in Malaysia

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Abstract

The cabbage webworm, *Hellula undalis* (F.) is a major pest of cruciferous vegetables in the lowlands of Malaysia. The common host plants include cabbage, radish, cauliflower, Chinese kale, Chinese mustard and the *Cleome* weed spp. Damage by cabbage webworm is particularly serious on cabbage because damage on the shoot by a single larva can cause either death of the plant or production of unmarketable multiple heads. The critical period of damage is usually from transplantation to the heading stage of cabbage. Generally, higher populations of CWW on cabbage coincided with the drier periods of the year, and low populations during the wetter ones. Within a single season, peak population generally occurred around 40 days after transplanting. Under laboratory conditions, the total developmental period from egg eclosion to adult emergence was about 26 days. Incubation period was about 3 days and the larval period about 14 days. There were usually five instars. The pupal period lasted 8.5 days. Adult longevity was about 7 days for both sexes and the shape of the survivorship curve was a Slobodkin's Type 1. The mean number of eggs laid per female was 175 and the mean egg per day per female was 27. Cabbage webworm was effectively controlled by shoot-tip treatment using *Bacillus thuringiensis* applied once a week until head formation. So far, two species of parasitoids have been recorded but their incidence was low.

Introduction

Many insect pests attack cruciferous vegetables in Malaysia (Yunus and Ho 1980). Among those of growing concern is the cabbage webworm (CWW), *Hellula undalis* (F.) (Lepidoptera:Pyralidae). CWW was first recorded in Malaysia in 1922 (Ooi 1979), but little research was done on this pest until the early 1980s. However, with the introduction of the heat-tolerant hybrid lowland cabbage varieties in 1973, CWW emerged as a major pest that warranted serious attention (Lim et al. 1990). Currently, CWW is an important pest on crucifers in almost all lowland areas in Malaysia, including Sarawak and Sabah. Ooi (1979) reported its occurrence as a minor pest in the Cameron Highlands (altitude 1525 m) where the temperatures are relatively cooler. However, there has been no confirmation on this in subsequent studies. Sachan and Gangawar (1980) suggested that a decrease in importance of CWW in India was related to the increase in altitude at which crucifers are increasingly being grown.

Ooi (1979) suggested that CWW could probably have been introduced into Malaysia through commercial activities because it lacked a good complement of natural enemies and crucifers are its natural hosts. This was the case for the diamondback moth (Tan and Lim 1985). Our observations on harvested cabbage heads, where we found live CWW larvae even into the 18th folded cabbage leaf, tended to lend some support to this suggestion.

Nature of Damage and Host Plants

The importance of CWW on crucifers, particularly cabbage, is underlined by the fact that a single larva, by virtue of its boring in the shoot, could either cause death to the young plant or the formation of unmarketable multiple heads on relatively older plants. This means that it is not possible to advocate specific chemical control measures for CWW, and that the control should be, by and large, preventive. In the field, a low population of larvae could cause significant losses, and in untreated cabbage, losses could go as high as 99%. Although the larva is present throughout the crop, it is severe only during the period between transplanting and the heading stage of cabbage.

In Malaysia, besides cabbage (*Brassica oleracea* var. *capitata*), CWW infests other crucifers such as cauliflower (*B. oleracea* var. *botrytis*), radish (*Raphanus sativus*), Chinese kale (*B. alboglabra*) and Chinese mustard (*B. juncea*) (Yunus and Ho 1980). Noncruciferous weeds such as *Cleome* spp. and *Hygrofolia salicifolia* were also found to be natural hosts for this insect. Our studies in the glasshouse showed that CWW prefers Chinese mustard over cabbage and radish. In terms of oviposition, the caged moth laid more readily on Chinese mustard and radish compared to cabbage.

Biology and Ecology

The egg of CWW is oval, about 0.44 mm in length and 0.32 mm in diameter. It is white when freshly laid but later turns slightly pinkish and then brownish-red just before hatching. Eggs are laid either singly or in rows of 2 or 3. The developmental durations of the various stages of CWW on cabbage are summarized in Table 1. The egg incubation period was about 3 days and mean egg viability was 60%. There were generally five instars on cabbage. The first instar, which usually mined the leaf, lasted about 3 days. The second instar ranged from 1 to 3 days, third instar from 2 to 5 days, fourth instar from 2 to 3 days and fifth instar from 3 to 5 days. There was a short prepupal period of about 1 day. Pupation normally occurred in the soil within a pupal case or in leaf debris. In the laboratory, pupation occurred at the sides of the breeding cage or the sides of the glassware where it is bred. The mean pupal period was 8.5 days. The total developmental period was about 26 days when bred on cabbage. The sex ratio of adults that emerged from field-collected larvae bred in the laboratory was 0.57.

The adult is a grey moth measuring 6-7 mm in length with a wing span of 14-15 mm. The forewing has wavy markings with a distinct kidney-shaped spot at about one-third the length from the apex. In the newly emerged female, these markings were relatively darker than the male. Further, in the female, the terminal segment of the abdomen is long and pointed whereas in the male it is relatively blunt. Adult longevity was about 7 days for both sexes and the shape of the survivorship curve was a Slobodkin's Type 1 (Fig. 1). In most adults, oviposition generally

Table 1. Development of CWW on cabbage under laboratory conditions (28 ± 2°C, 70-90% RH).

Stage	Days (mean ± SD)
Egg	2.89 + 0.41
Larva	
Instar 1	3.00 + 0.00
Instar 2	2.20 + 0.75
Instar 3	3.20 + 0.98
Instar 4	2.00 + 0.63
Instar 5	4.00 + 0.89
Pupa (+ prepupa)	8.50 + 0.58
Total developmental period (egg to adult emergence)	26.00 + 1.15

commenced within 24 hours and might last from 3 to 10 days. The mean number of eggs laid per female was 175 and the mean eggs per day per female was 27. The peak oviposition time was on the second day after emergence (Fig. 1).

Field studies on cabbage cultivated continuously did not reveal any obvious yearly trends. However, peak populations generally coincided with the drier periods of the year, i.e from February to April and June to July whereas the population was low during the wetter periods from September to December. In the field, on a single cabbage crop, the peak population of CWW normally occurred around 40 days after transplanting. The distribution of the larvae per plant is shown in Fig. 2. The mean larval population was normally one per plant with a maximum of nine. Aziz noted that before heading, most of the larvae (88.5%) were found on the peripheral leaves whilst 11.5% were found on the shoot region of cabbage.

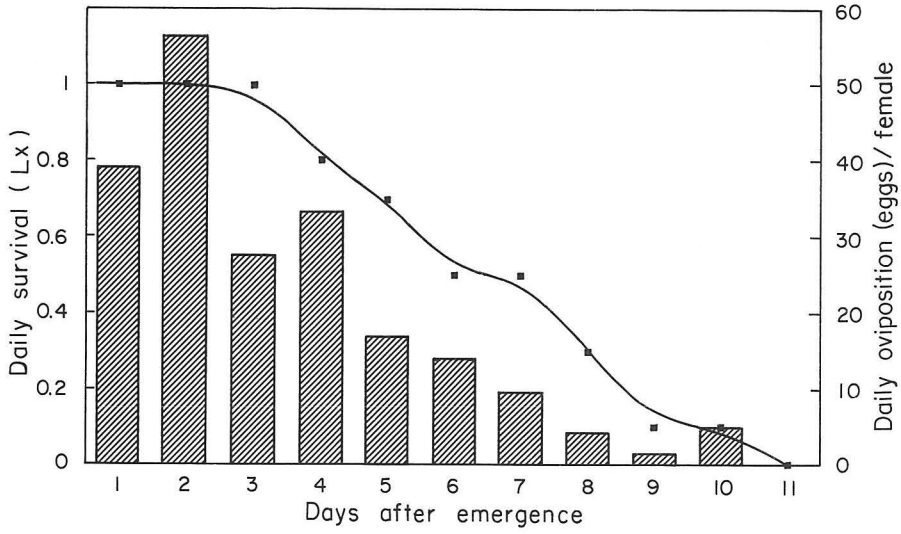

Fig. 1. Survival and oviposition pattern for CWW under laboratory conditions (28 ± 1 °C; 70-90% RH).

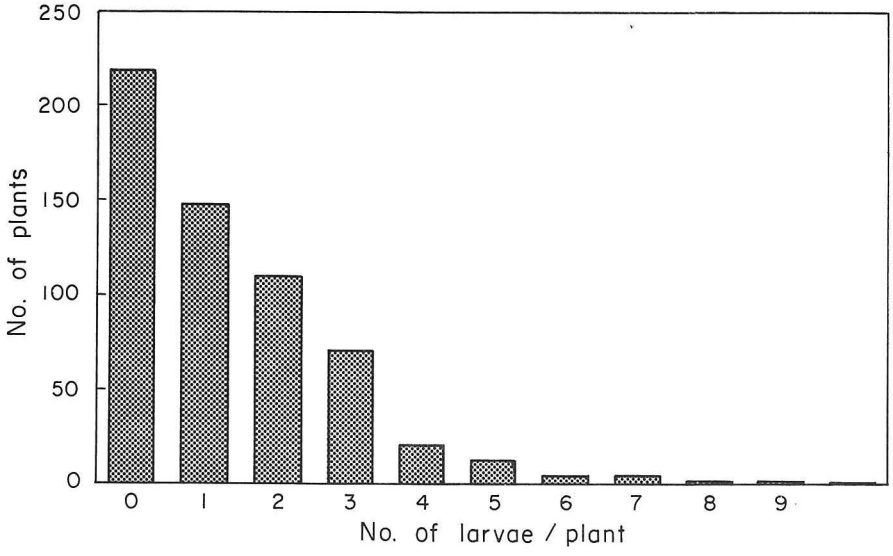

Fig. 2. Distribution of CWW larvae on cabbages.

Control Measures

Insecticides

This is currently the only effective method of control for CWW in the field. Ng (1980) evaluated eight insecticides against CWW on cauliflower and found that sprays of trichlorfon 95 (0.02% ai) and sulprofos (0.10% ai) gave 100% control at 1 and 6 days after initial spraying. There was no significant difference between the different rates tested. Following this MARDI (1981) reported that *Bacillus thuringiensis* Berliner, *B. thuringiensis* + granulosis virus and decamethrin-treated cabbage plots had significantly lower populations and higher marketable heads than in the untreated check plots. However, in the same trial, the virus and dimethoate-treated plots had relatively high populations of CWW (Table 2) vis-a-vis the control plot. In a later trial (MARDI 1986), nine insecticides were evaluated. It was found that CWW can be effectively controlled by shoot treatment carried out at the early stage of plant growth (1-1.5 months after transplanting). The effective insecticides were permethrin, abamectin, teflubenzuron, chlorfluazuron, triflumuron, phenthoate, exthofenprox and l-cyhalothrin. Further screening was done in 1987 using eight insecticides (MARDI 1987), which included abamectin, profenofos, cypermethrin, triazophos, etrimfos, benzoylurea and permethrin. Abamectin was more effective than the other insecticides. In abamectin-treated plots only 2% of the plants were damaged compared to 42.2% and 65.0% in the two control plots. Permethrin which was effective in 1987, however, registered the highest percent damage (15.7%) and the lowest yield (18.6 kg/plot) amongst the insecticide-treated plots (Table 3). Besides permethrin, the use of the other insecticides

Table 2. Efficacy of insecticides and two microbials against CWW in cabbage plots at Jalan Kebun, Selangor, Malaysia.

Treatment	No. insects[a]	No. heads per plot (mean)	% marketable heads
Granulosis virus (GV)	256 c	7.8 b	0.0 b
B. thuringiensis (Bt)	65 d	15.3 a	51.3 a
GV + Bt	71 d	14.5 ab	62.5 a
Deltametrin	84 d	16.8 a	65.0 a
Dimethoate	419 a	8.0 b	0.0 b
Untreated control	330 b	10.3 ab	6.3 b

[a]Population mean for all sampling dates. Numbers followed by the same letter, for a given column, are not significantly different according to DMRT (P = 0.05). Source: MARDI 1981.

Table 3: Evaluation of insecticides for the control of CWW on lowland cabbage based on shoot-tip treatment.

Treatments	No. Larvae per 10 plants (mean)	% damaged plant/plot	Weight of marketable heads (kg/plot)
Abamectin	0.01 c	2.0 c	28.2 a
Profenofos	0.01 c	8.4 c	22.0 b
Cypermethrin	0.02 c	11.0 c	21.6 b
Triazophos	0.02 c	12.5 c	21.5 b
Etrimfos	0.03 c	12.7 c	20.5 b
Benzoylurea	0.03 c	15.0 c	20.1 b
Permethrin	0.03 c	15.7 c	18.6 b
Control A	0.15 b	42.2 b	12.1 c
Control B	0.27 a	65.0 a	5.1 d

Source: MARDI 1987.

such as triazophos, profenofos, fenthion, methamidophos, the IGRs and cypermethrin currently seemed to be fraught with difficulties as they do not provide adequate control of CWW. This suggested that there could be resistance development by this insect to these insecticides. However, this is yet to be confirmed in the laboratory. Fortunately, *B. thuringiensis* is still very effective against this pest. In a recent trial (MARDI 1989), it was found that Thuricide (*B. thuringiensis*, 16,000 IU/mg) and Florbac (*B. thuringiensis*, 8500 IU/mg) shoot-tip treatment gave very good control against CWW. Insecticide application is done weekly starting from 3 to 5 days after transplanting to at least the first 4 weeks until heading. In severe cases, twice weekly applications are done.

Biological control

In Malaysia, there is little information on the natural enemies of this pest. Tan (pers. comm.) recorded an ichneumonid *Trathala flavoorbitalis* as a larval/pupal parasitoid. In field trials, we found a braconid *Bassus* sp. (indet.) emerging from the larva. However, its incidence in the field based on percentage parasitism is low (less than 15%). Lim (1982) reported that *Cotesia plutellae* parasitized CWW under laboratory conditions and attained successful pupation. However, no adult emergence was noted. In the laboratory, we observed a protozoan disease affecting the larval population, especially when the larval food substrate (cabbage leaves) was too moist. Unfortunately, not much is known about this disease.

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9

Biology and Control of *Crocidolomia binotalis* in Indonesia

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Abstract

The diamondback moth, *Plutella xylostella* (L.), is the most important pest of crucifers in Indonesia. In most highland vegetable areas, this pest has been effectively controlled by the ichneumonid parasitoid, *Diadegma semiclausum* Hellen. However, the cabbagehead caterpillar (*Crocidolomia binotalis* Zeller) which is the secondary pest of cabbage, may become a serious problem, particularly during the dry season. Results of laboratory and field studies of *C. binotalis* during the last 10 years are reviewed and discussed in this paper. Under laboratory conditions (26-33°C and 54-87% RH), the life cycle lasted about 28 days (26-32 days). This period increased from 30 to 41 days at lower temperatures (16-22°C). The egg incubation period lasted for 4 days (3-6 days). There are five larval instars with the mean duration of 14 days (11-17 days). The pupation period took about 10 days (9-13 days). A female moth may lay on an average 300 eggs (68-590 eggs) in its lifetime; 92% (69-100%) of the eggs will hatch. Two larval parasitoids were tentatively identified as *Inareolata argenteopilosa* Cam. (Ichneumonidae) and *Sturmia inconspicuoides* Bar. (Tachinidae). Rates of parasitization were low throughout the sampling period. Larval population increased starting from 2 weeks after planting, peaked at 8-10 weeks after planting and declined thereafter up to harvest time. Abundance of *C. binotalis* larvae was negatively correlated with rainfall. Counts of the immature stages of *C. binotalis* showed that the distribution of larval populations followed a negative binomial pattern. Studies have also been conducted to evaluate efficacy of insecticides on *C. binotalis* larvae and their effect on the fecundity of female moths. In line with the development and implementation of integrated control of *C. binotalis*, control threshold level needs to be determined. Research along this line is still underway.

Introduction

Since 1973, it has been known that the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), and cabbagehead caterpillar (CHC), *Crocidolomia binotalis* Zeller (Lepidoptera: Pyralidae), are harmful and economically important caterpillars on cabbage in Indonesia. If suitable control is not undertaken, especially in the dry season, the yield loss caused by both insect pests together may be up to 100% (Sudarwohadi 1975). Since that time, considerable research has been done on bioecology and control of DBM, while less attention was given to CHC. The success of biological control program of DBM by the introduced parasitoid *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae), does not mean that the pest problem on cabbage cultivation has been completely solved (Sastrosiswojo and Sastrodihardjo 1986). In some cases, CHC may become an even more serious problem, particularly during the dry season and in the areas where insecticide use is less. In spite of the economic importance of CHC on cabbage in Indonesia, very little is known of its life history, population ecology

and proper control strategy. This paper reviews the research work carried out on CHC since 1981.

Biology

Host Range

CHC infests wild and cultivated crucifers, including cabbage (*Brassica oleracea* var. *capitata* L.), cauliflower (*B. oleracea* var. *botrytis* L.) and Chinese cabbage (*B. campestris* spp. *pekinensis*). CHC occurs to a lesser extent on turnip (*B. rapa* var. *rapa* L.), sprouting broccoli (*B. oleracea* var. *botrytis* L. subvar. *cymosa* Lam.) and radish (*Raphanus sativus* var. *hortensis*) (Gunn 1925, Kalshoven 1981). It has also been reported that mustard (*B. juncea* Coss.), watercress (*Nasturtium officinale*) and an ornamental crop *Clerodendron fragranspeniflorum* Vent. are host plants of CHC (Thayib 1983).

CHC exhibits a marked preference for ovipositing on turnip rape (*B. campestris* ssp. *oleifera* Metzg. Sinisk) and pak choy (*B. chinensis*) (Setiawati 1989).

Life History

The biology of CHC has been studied in the laboratory, including its life cycle and behavior (van den Oever 1973; Othman 1982; Thayib 1983; Setiawati 1989).

Egg stage: The eggs are light green and usually oviposited on the underside of cabbage leaves. Before hatching, the color of the eggs changes to orange, yellowish-brown and dark-brown. The eggs are laid in overlapping masses of 9-120 eggs with an average of 48. The size of egg clusters ranges from 1.0 × 2.0 mm to 3.5 × 6.0 mm with an average of 2.6 × 4.3 mm.

The incubation period for eggs was 4 days (range 3-6 days) at 26.0-33.2°C. The percentage of hatching is 92.4% (range 69.2-100%) (Othman 1982).

Larval stage: The newly hatched larvae are gregarious with black heads and light green body and dark spots. The larvae are characterized by whitish longitudinal stripes, three dorsally and one on each lateral side. The fully grown larvae are 15-21 mm in length.

There are five larval instars. The duration of each successive instar was 2.6 days (range 2-4 days), 2.4 days (range 1-3 days), 2 days (range 1-3 days), 2.3 days (range 1-5 days) and 4.7 days (range 3-7 days), respectively. The total larval period extends to 14 days (range 11-17 days) at 26.0-33.2°C and 54.1-87.8% RH (Othman 1982). However, van den Oever (1973) reported that the larval period varied from 10-14 days at 16-22.5°C and 60-85% RH (Table 1).

The damage caused by CHC on cabbage can be a serious problem because the caterpillars prefer the young leaves and growing point which are succulent, and often devour it completely. This trend is clearer when the larvae enter the third instar stage. If the caterpillars attack cabbage plants during the head formation stage, they will penetrate into the head, make tunnels and the crop will rot.

Pupal stage: Usually pupation takes place on the soil surface. The pupa is yellowish brown and later becomes dark-brown. The size of pupa is about 3 mm wide and 10 mm long. The pupal stage ranged from 9 to 13 days with an average of 10 days at 26.0-33.2°C and 54.1-87.8% RH (Othman 1982). Van den Oever (1973) reported that the pupal period was 13-18 days at 16-22.5°C and 60-85% RH.

Adults: The female moths emerge about 1 day before the males (van den Oever 1973). The adults have black thorax and reddish-brown abdomen. The females bear a curved ovipositor and are generally larger than the males. The moths are nocturnal, but are not attracted by artificial

Table 1. Life cycle of CHC in Indonesia.

Item	26.0 - 33.2°C; 54.1 - 87.8% RH[a]	16.0 - 22.4°C; 60.0 - 85.0% RH[a]
Oviposition period (days)	8.5 (3-19)	several days
Egg incubation period (days)	4.4 (3-6)	(4-5)
Egg viability (%)	92.4 (69-100)	almost 100%
Larval period (days):		
1st instar	2.6 (2-4)	(3-4)
2nd instar	2.4 (1-3)	(2-3)
3rd instar	2.0 (1-3)	(2-3)
4th instar	2.3 (1-5)	(3-4)
5th instar	4.7 (3-7)	–
Total	14.0 (11-17)	(10-14)
Pupal period (days)	10.3 (9-13)	(13-18)
Adult longevity (days)		
Mated male	15.9 (6-30)	–
Mated female	15.2 (8-26)	–
Life cycle (days)	28.3 (26-32)	(30-41)
Sex ratio (male: female)	0.9:1.0	1.0:1.0

[a]Numbers in parentheses indicate range. Adapted from van den Oever (1973), Othman (1982) and Thayib (1983).

light. During the day they hide under the cabbage leaves and when disturbed they will fly briefly. The color pattern of the male forewings is sharper than the females. The males can be easily recognized by a tuft of dark hairs at the anterior margin on both of the forewings. The males have longer body (11.4 mm) than the females (9.6 mm). Visually, the females have larger abdomen than the males.

Mating, oviposition, and fecundity: Thayib (1983) reported that the sex ratio of CHC was male:female, 1:1. The moths mate after 2-3 days from emergence. In the field, mating always happens around midnight until early morning. In the laboratory, mating may occur during the day in dark places after about an hour's darkness. Oviposition usually takes place at night, and at least starting from 1 day after copulation for 2-4 days.

Othman (1982) reported that the females are able to deposit 2-21 egg clusters containing a total of 60-598 eggs when fed with honey and 1-13 egg clusters containing 11-294 eggs when no honey was given. The oviposition period was 3-10 days when honey was given and 1-7 days when not supplied with honey. The life span of mated males and females lasts for 6-30 days and 8-26 days when honey was given, and 3-23 days and 3-14 days when not provided with honey. Thus, the role of diluted honey (honey:distilled water = 1 : 1) is very important in mass-rearing of CHC.

Under laboratory conditions (16-22.5°C and 60-85% RH), van der Oever (1973) reported that the total life cycle of CHC ranged from 30-41 days. However, Othman (1982) mentioned that at 26.0-33.2°C and 54.1-87% RH, the duration of one generation CHC ranged from 26 to 32 days (average 28 days) (Table 1).

Natural Enemies

Parasitoids: Some parastioids of CHC larvae collected from Sindanglaya (Segunung areas) from 1927 to 1931 and preserved in the museum of the Central Research Institute For Agriculture (now Bogor Research Institute For Food Crops) are: *Sturmia inconspicuoides* Bar. (Diptera:Tachinidae), *Inareolata argenteopilosa* Cam. (Hymenoptera:Ichneumonidae), *Mesochorus* sp., *Atrometus* sp. and *Chelonus tabonus* (Sonan). According to van den Oever (1973), *I. argenteopilosa* attacks the second or third instar larvae, while *S. inconspicuoides* attacks the third or fourth instar larvae. The rate of parasitism of both species was low, viz. 1.6% for

I. argenteopilosa and 4.4% for *S. inconspicuoides*. Othman (1982) reported that *I. argenteopilosa* was more predominant than *S. inconspicuoides*. From field-collected larvae, she found that the percentage of parasitism of the *I. argenteopilosa* ranged from 1.1 to 7.2%, whereas the *S. inconspicuoides* ranged from 0 to 4.1%. Under laboratory conditions, *I. argenteopilosa* could parasitize the first to the third larval instars. However, it seemed that the parasitoid preferred the 2-day-old larvae as they had softer and thinner skin than older larvae.

The percentage of parasitism decreased with age of larvae. It seemed that the percentage of parasitism also decreased as the host population increased. The highest rate of parasitism was 31.3% when the parasitoid-host ratio was 1:10. The duration of the life cycle of *I. argenteopilosa* under laboratory conditions (26.0 - 33.2°C and 54.1 - 87.8% RH) was 16-21 days (Table 2). Adult longevity for both mated male and female was 3-17 days.

Table 2. The development periods of *I. argenteopilosa* on 4 - 5-day-old CHC larvae.

Stage		Mean (range) duration (days)
Egg and larva period, on:	4-day-old host larvae	10.3 (8-13)
	5-day-old host larvae	8.3 (7-9)
Pupal period		8.0 (7-9)
Total life cycle, on:	4-day-old host larvae	18.2 (18-21)
	5-day-old host larvae	16.7 (16-17)

Source: Othman (1982)

Predators: Van den Oever (1973) reported that by observations in the field, only predation by a black beetle larva (Coleoptera: Carabidae) was noticed. The predation capacity was unknown.

Pathogens: During a 2-year study on the bionomics of CHC, Thayib (1983) collected diseased larvae affected by bacteria and fungi. Results from isolation and microscopic examination indicated that the dead specimens were infected with *Proteus* spp., *Achromobacter* sp. and *Bacillus* sp. (bacteria), and fungi from the genera *Aspergillus*, *Fusarium* and *Penicillium*. Reinfection trials showed that the pathogen virulence was not consistent, especially when the relative humidity was not high.

Ecology

Geographical distribution

CHC is a common pest of cruciferous crops with a worldwide distribution in tropical and temperate regions. Its area of distribution is reported as South and Southeast Asia, Australia, South Africa, Tanzania and the Pacific Islands (Dammerman 1929; Kalshoven 1981).

Seasonal incidence

Seasonal incidence of CHC on cabbage has been studied in Indonesia at Segunung (altitude 1100 m) (van der Oever 1973; Sudarwohadi 1975; Thayib 1983). The oviposition peaks in February, May and July-August. High buildup of larval populations was in March, June and August (Fig. 1). This coincided with the drier part of the year at Segunung. This also indicates that there is a negative correlation between the CHC populations with rainfall; higher rainfall increases insect mortality.

Although it was not very clear, it may be assumed that there are two oviposition peaks during one growing season (Fig. 1). This means that at least there are two generations of CHC during one growing season. In general, the study indicated that within 90 days of cabbage growing period, the population of CHC larvae tends to increase starting from 2 weeks after planting, peaks at 6-8 weeks later and declines thereafter up to harvest time.

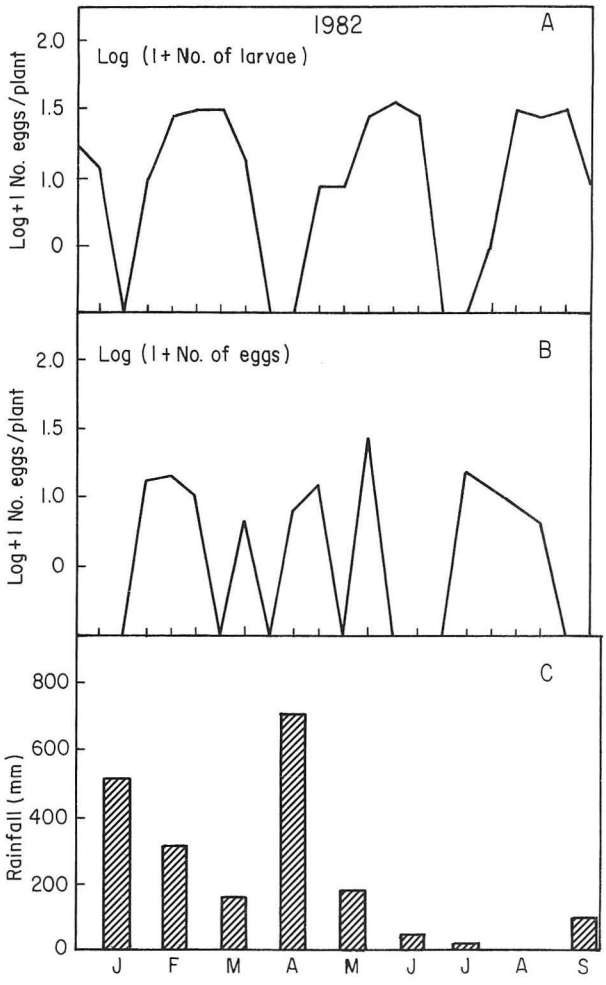

Fig. 1.
(A) Number of CHC larvae/ prepupae per plant, (B) Number of CHC eggs per plant, and (C) Monthly rainfall.

Population distribution pattern

The pattern of population distribution of CHC larvae on cabbage was recently investigated at Lembang by Tohidin (1990). CHC larvae tend to aggregate on cabbage plants. Their spatial distribution on cabbage has the characteristic of being contiguous and follow the negative binomial distribution. The optimal sample size for CHC larvae was 54 plants with one quadrant of sample unit per plant.

Chemical Control

Since up to the present there is no other alternative control for CHC, chemical control is the most common method. Various chemical and microbial insecticides have been recommended for the control of CHC on cruciferous crops.

Commercial preparations of *Bacillus thuringiensis* Berliner (Dipel WP, Bactospeine WP and Thuricide HP) at 1.0 - 2.0 kg formulated product per hectare were reported effective against CHC (Sudarwohadi et al. 1973; Sastrosiswojo 1987; Setiawati and Sastrosiswojo 1989).

Chlorfluazuron and teflubenzuron (insect growth regulators) were effective against CHC at 40 g AI/ha (Sastrosiswojo 1987; Soeriaatmadja and Duskarna 1990). Based on the laboratory study, it was found that *B. thuringiensis* (Dipel WP) and chlorfluazuron were more effective against early instar than late instar larvae of CHC (Setiawati and Sastrosiswojo 1991, in press). The LC_{50} values against second instar larvae of CHC were 42 ppm for *B. thuringiensis* and 424 ppm for chlorfluazuron, whereas against fourth instar larvae they were 755 and 1068 ppm respectively. Chemical insecticides reported to be effective against CHC were permethrin, cypermethrin, decamethrin, profenofos, prothiophos and acephate (Sastrosiswojo 1987; Soeriaatmadja and Duskarno 1990). However, laboratory studies proved that acephate and permethrin may cause a resurgence of *C. binotalis*. Acephate and permethrin induced increases of fecundity of adults by 98 and 93%, respectively (Setiawati 1990). The reasons and mechanism of this resurgence are not fully understood.

Integrated Pest Management Approach

The development and implementation of integrated control of CHC cannot be separated from a DBM program. At present, *D. semiclausum* is an important biological control agent of DBM in Indonesia, especially in areas where it is well established (Sastrosiswojo and Sastrodihardjo 1986). Integration between biological control and the use of selective insecticides based on the control threshold of DBM (0.5 larva/plant) will reduce the amount of insecticde usage by 40-60% (Sastrosiswojo 1987). Thus, the population of CHC may increase, since so far there is no effective biological control agent for CHC. Some alternative control stra-tegies that might be implemented are as follows:

(1) The most rational and primary step is to develop control threshold of CHC. Monitoring may be based on the population of CHC eggs or second-third instar larvae, or visual damage threshold as suggested by Srinivasan (1984 cited by Chelliah and Srinivasan 1986). Research along this line is still being undertaken at LEHRI, Lembang.

(2) Superimposition of damage threshold on the intercrop combination of one row of cabbage and one row of tomato is also advocated as an effective alternative approach to reducing cabbage yields significantly (Chelliah and Srinivasan 1986).

(3) The encouragement of biological control in any IPM program is also important. Although the larval parasitoid *I. argenteopilosa* occurs in Indonesia, the level of parasitism is low. Therefore exotic parasitoids should be introduced to complement the existing ones. Another possibility is the use of pathogens such as entomogenous fungi or viruses. These pathogens should be explored in the country or if possible introduced into Indonesia.

(4) There is doubt that chemical control is still an important key component in an IPM approach. As far as CHC is concerned, the use of insecticides that do not harm or are less toxic to *D. semiclausum* but effective against DBM and CHC is strongly recommended.

(5) Other factors to be considered in the IPM program are monitoring system, sampling technique and sample size. The use of sex pheromone might be important, both for monitoring or mass trapping.

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SEX PHEROMONE

10

Pheromonal Control of Diamondback Moth in the Management of Crucifer Pests

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Abstract

The effect of delayed mating on the reproductive potential of the diamondback moth, *Plutella xylostella* (L.), population was evaluated in the laboratory. The insecticide-resistant diamondback moth could be controlled by synthetic sex pheromones which are not harmful to beneficial species. However, the pheromones had no effect on other pests like aphids and common cabbageworm. Predators crawling on the ground, like lycosid spiders, played an important role as a biotic mortality agent of immature stages of diamondback moth. The chitin synthesis inhibitors that are selective insecticides were very effective on caterpillars like the common cabbageworm. Pheromone and chitin synthesis inhibitors which are harmless to beneficial arthropods are regarded as main chemicals acceptable in management of crucifer pests.

Introduction

The main drawbacks in insecticidal control of diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), are: (1) development of insecticide resistance; (2) resurgence of the insect after applications of insecticide; (3) nonselective killing of harmless and beneficial species (Nemoto 1986).

Synthetic sex pheromones have been utilized to suppress insect pest populations through either disruption of communication between the sexes or mass trapping of adult males. The efficacy of the pheromone is frequently measured by making comparisons between the percentage of tethered virgin females that mate in treated and control plots (Nemoto et al. 1985). In this paper, we present a converted Kiritani and Kanoh's equation (ER) and attempt to evaluate the effect of delayed mating on the reproductive potential of the DBM population by laboratory experiment. A disruption experiment was conducted in a commercial grower's field to evaluate efficacy of synthetic sex pheromones in controlling DBM.

Nemoto (1986) reported that according to immunological tests lycosid spiders are important as biotic mortality agents of DBM. The role of predator or parasite in this experiment was evaluated by artificially excluding them from the estimation of DBM mortality.

The control of only one species of pest is meaningless for crucifers that require simultaneous protection from other pests. The effect of insecticides on these pests was evaluated.

Evaluation of delayed mating DBM population

The possible role of a pheromone-induced delay in mating on the reproduction of the oriental tea tortrix, *Homona magnanima* Diakonoff, has been examined by Kiritani and Kanoh (1984), who considered that the delayed mating or fertilization on the part of females might reduce their fecundity through shortening their reproductive period and aging. They proposed an equation for the expected reproduction (ER) of a t-day-old female.

A high percentage of mating inhibition, for example 90%/night, however, does not result in the same degree of suppression of the target population if the mean longevity of the adults exceeds 1 day. If the virgin females lived for only 1 day, a 90% mating inhibition/night would result in 10% of the females being mated, while up to 65% ($1 - 0.9^{10} = 0.65$) of the females would eventually be fertilized if they lived for 10 days. On the other hand, the delay in mating reduces total fecundity through shortening the female reproductive period. Yamada (1979) observed that most of the newly emerged females mated on the first night of their emergence and began to lay eggs on the following night. Unmated females lay a few eggs. The effect of delayed mating on various reproductive traits is presented in Table 1. Fecundity, viability of eggs and oviposition period all decreased with an increase in number of days elapsing before pairing after emergence.

Table 1. Effect of delayed mating on the reproductive traits of DBM.

Days after emergence and before pairing	No. of pairs	No. (and %) of pairs mated	No. of eggs laid/ovipositing female	No. of fertilized eggs/ovipositing female	Expected reproduction (RER)
0	20	17(85)	108.1 ± 8.8a	102.9 ± 28.9e	87(95)
2	19	18(95)	100.9 ± 10.9ab	97.1 ± 10.9e	92(100)
4	20	14(70)	79.6 ± 10.0bc	77.9 ± 10.1ef	41(44)
6	15	10(67)	71.8 ± 11.8c	60.3 ± 13.9f	21(23)
8	11	8(73)	61.8 ± 15.3c	51.1 ± 15.6f	15(16)
11	14	8(57)	73.9 ± 15.0bc	52.4 ± 16.8f	14(15)
Unmated control	28	–	13.6 ± 24.3 d	–	0(0)

The expected reproduction (the total number of viable eggs that could be laid during the female lifespan) of a t-day-old female which mated first on the tth day after emergence can be expressed by the following equation (Kiritani and Kanoh 1984):

Expected reproduction of a t-day-old female (ER) = Percentage successful mating × Survival rate until tth-day × Total no. of viable eggs deposited

This may be converted to the following equation:

$$ER = \text{Percentage successful mating} \times \Sigma \text{ the age-specific survival rate of female adults} \times \text{age-specific fecundity}$$

Successful mating refers to mated females that lay fertilized eggs. All mated females laid fertilized eggs in the present experiment. The relative expected reproduction (RER) of the female mated t days after emergence is then calculated (Table 1). The effect of delayed mating on ER becomes highly significant when the delay exceeds 6 days. This effect would be intensified under natural conditions, where adult survival would be influenced by weather, predators and other factors.

Utilization of synthetic sex pheromones for DBM control aims to reduce the number of fertilized females in a given area. The effect of pheromone on the pest population therefore depends on the extent to which the pheromone application inhibits mating of virgin females. The realized RER (see the 4th column in Table 2) of the treated population at various levels

of inhibition was calculated to evaluate the effect of this inhibition on the reproduction of the DBM population.

The cumulative percentage of mated females and the realized RER of populations subjected to various levels of mating inhibition are shown in Table 3. If it is assumed that the life span of females is invariably 12 days, then mating inhibition as high as 72% will have little influence on the population size of the following generation. Ninety percent inhibition is expected to reduce the target population to 41% of the untreated population, assuming no immigration from outside of the plot. A similar conclusion has been reached by Kiritani and Kanoh (1984) for *H. magnanima* and Nakasuji and Fujita (1980) for *Spodoptera litura* (F.) populations by means of computer simulations.

Comparison between the cumulative mating percentage and the realized RER shows that the initial reduction of RER occurs at a lower level of daily mating inhibition than the cumulative mating percentage (Table 3). This is because realized RER involves the effect of delayed mating on reproduction. Delayed mating also leads to a delay in oviposition or lengthening of the preoviposition period. Although this factor has not been considered in the present discussion, it may play an important role in slowing down the population growth of insects under certain conditions. From the practical point of view, the optimum level of mating inhibition will depend on the cost: benefit aspects of the relationship between the number of pheromone sources required and the necessary level of mating inhibition. The latter is also a function of pest density and the reproductive traits of the species.

It can be concluded that the effect of sex pheromone on the target DBM population depends not only on the direct effect of mating inhibition, but also on the indirect effects of delayed mating which reduces ER. A high level of mating inhibition (more than 90%) and/or other mortality agents would be required for a substantial reduction of DBM populations.

Table 2. Cumulative percentage of mating females and the realized relative expected reproduction (RER) for the population when mating of females is inhibited.

Pivotal age of female	RER (A)	Daily rate of virgin females (B)	Realized RER (C) = (A).(B)
0.5	95	0.10	9.5
1.5	98	0.09	8.8
2.5	100	0.08	8.1
3.5	79	0.07	5.8
4.5	44	0.07	2.9
5.5	30	0.06	1.8
6.5	23	0.05	1.2
7.5	18	0.05	0.9
8.5	16	0.04	0.7
9.5	16	0.04	0.6
10.5	15	0.03	0.5
11.5	15	0.03	0.5

Lonegvity of females and the level of daily mating inhibition are assumed to be 12 days and 90%, respectively.

Table 3. Cumulative percentage of mated females and the realized RER in a hypothetical population where all females live for 12 days under various levels of mating inhibition.

Daily level of inhibition (%)	99	98	97	96	95	94	90	80	70	60	40	20	0
Cumulative % of mated females	11[a]	22	31	39	46	52	72	93	99	100	100	100	100
Realized RER of the population	5	10	17	19	24	28	41	65	79	87	94	95	100

[a] $1-(0.99)^{12} = 0.11$.

Pheromonal Control of DBM in the Field

A disruption experiment was conducted in a 5-ha field in Kawagoe, Saitama Prefecture, in 1989. Brassica greens (pakchoy), radish, taro and other vegetables were cultivated. In this area DBM has developed resistance to synthetic pyrethroids and other chemicals. Brassica greens were covered with netting to prevent physical invasion by flying adult pests. Twenty meters per 0.1 ha of KONAGA-CON (a rope-type dispenser containing synthesized sex pheromone for DBM in a 1:1 mixture of Z11-16Ald and Z11-16Ac (25 g AI/100m), and made by Shin-Etsu Chemical Co. Ltd.) were extended on the poles 40 cm high in the radish fields and on the netting tunnels in the brassica greens fields. The rope-type dispensers were arranged in a mosaic pattern in the 5-ha experimental fields. Throughout the season (June to October), the number of DBM males captured in the yellow sticky traps which were set as monitors in both the pheromone and pheromone+chemical (EPN) treatment fields was always lower than the traps in the control or only chemical (pyrethroid) treatment fields. The reduction of DBM larval density on radish leaves in both the pheromone and pheromone+chemical treatment fields was also apparent when compared with the density in the control or chemical (pyrethroids) treatment field (Fig. 1). The daily percentage mating inhibition using tethered virgin DBM females in the pheromone-treated field ranged from 50 to 100% (average 70%). Seventy percent inhibition is expected to reduce the target population to only 79% of the untreated population (see Table 3).

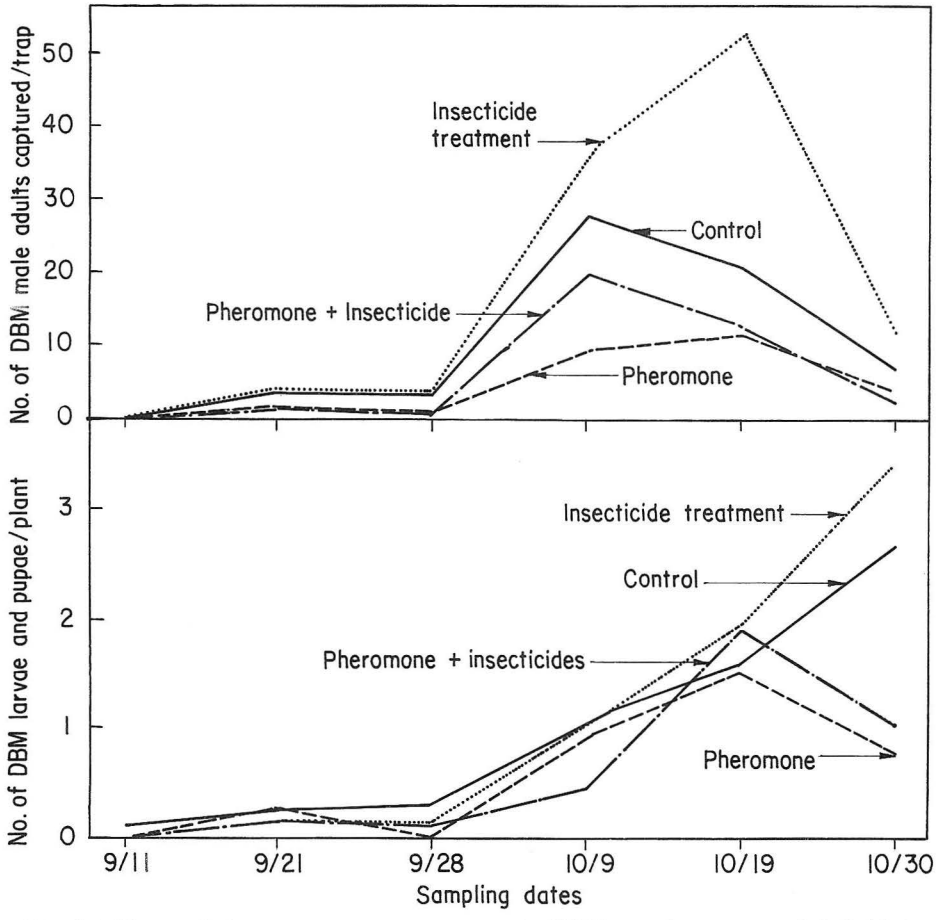

Fig. 1. Effects of pheromone treatments on the DBM populations in radish fields in 1989 at Kawagoe, Saitama, Japan.

But DBM could be controlled in the radish and brassica greens fields through the application of the synthetic sex pheromone which was harmless to beneficial arthropods. In this experiment, the pheromone treatment, however, had the most effect on DBM. We, therefore, thought that natural enemies played a more important role in the control of DBM in pheromone-treated fields.

Evaluation of Natural Enemies

The role of predator and parasite was evaluated by artificially excluding them from the estimation of DBM mortality. The brassica greens on which DBM eggs were laid in the laboratory within 24 hours were planted in cages in the cabbage field at Saitama Horticultural Experiment Station (SHES) during 1989-90. Cages (90 × 90 × 90 cm) were covered with mesh netting (0.2, 1.0, 1.4, 6.0 and 13.0 mm). Another cage (90 × 90 × 30 cm) was covered with vinyl film except the top and treated with a sticky substance on the upper edges of the vinyl film to exclude ground-crawling predators but to allow flying predators to invade the cage. The 0.2-mm-mesh cage excluded all predators and parasites. The 1.0- and 1.4-mm-mesh cages excluded predators but no parasites. The 6- and 13-mm-mesh cages excluded birds and/or predatory wasps but not ground-crawling predators. The larvae within the cages covered with 6 and 13 mm mesh and in the control plots decreased more rapidly than in the other cages (Fig. 2). This showed the importance of all natural enemies in reducing DBM population.

Nemoto (1986) reported the lycosid spiders were reduced through the application of methomyl, and were important biotic mortality agents of DBM based on immunological tests. These results suggest that ground-crawling predators like lycosid spiders play an important role as biotic mortality agents of immature stages of DBM.

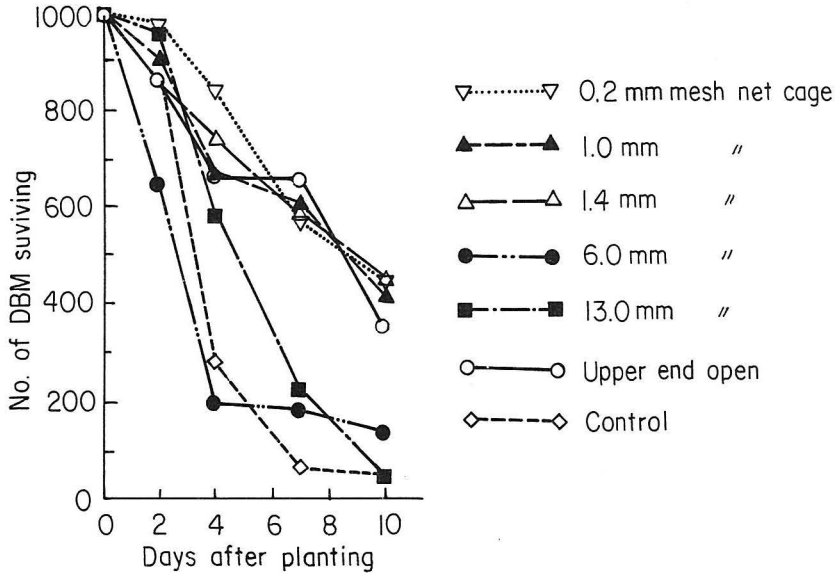

Fig. 2. Survivorship curves of immature stages of DBM (out of a thousand originally born) within the different mesh cage.

Effectiveness of different chemicals

The major pests of crucifers in Japan are DBM, common cabbageworm, *Pieris rapae crucivora* Boisduval, beet semi-looper, *Autographa nigrisigna* Walker, green peach aphid, *Myzus*

persicae Sulzer, and cabbage aphid, *Brevicoryne brassicae* L. The control of only one species of pest is meaningless for crucifers that also require simultaneous protection from other pests. The pheromone was able to control DBM, but didn't control other pests. If chemical insecticides were not sprayed, crucifer production was seriously affected by the common cabbageworm and cabbage aphid.

The effects of the chemicals with different modes of action on pests of crucifer vegetables are shown in Fig. 3. Thiocyclam was effective on caterpillars and aphids. This insecticide had good initial action against the pests, but its residual effect was weak. DBM and aphid populations increased again 20 days after spraying. Cypermethrin was effective against aphids and caterpillars, but not DBM. Chlorfluazuron and other chitin synthesis inhibitors, which are selective insecticides and are harmless to beneficial arthropods, performed well against caterpillars, but were ineffective for aphids.

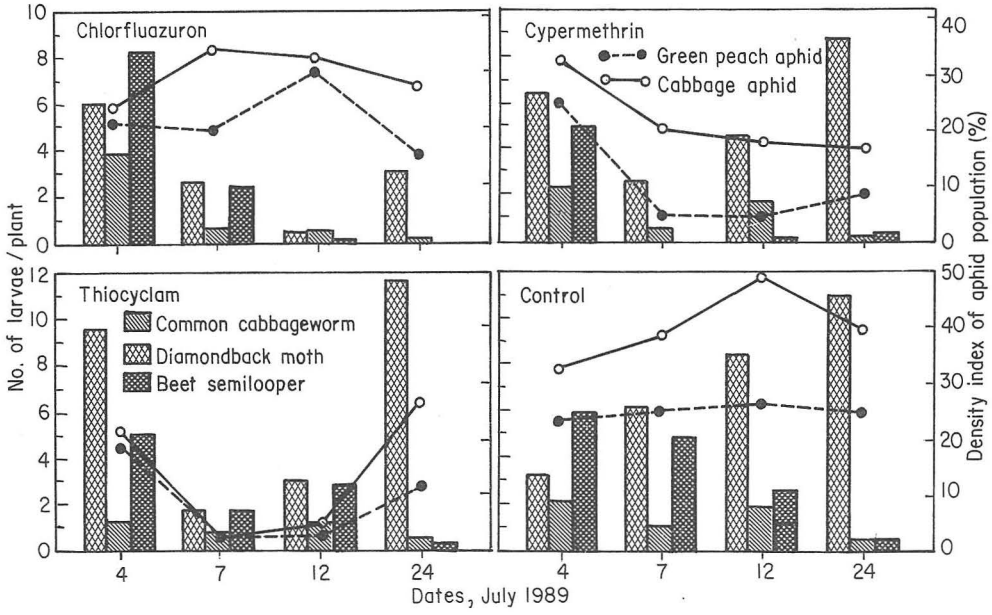

Fig. 3. Insect population densities after application of chemical at SHES in 1989.

Discussion

Insecticide resistance is most acute in DBM. The frequency of resistance in pest population is in large part a result of selection pressure from pesticide use (Georghiou 1980). Insect pests which were resistant to insecticides could be controlled by pheromones. The pheromone reduced the frequency of application of insecticides to control DBM, but the pheromones cannot control other pests. It is difficult for farmers not to spray chemical insecticides, because of the lack of economical alternative pest control methods for the other pests, except *S. litura*. Mass trapping of *S. litura* by synthetic sex pheromones was demonstrated successfully in 1978 in the area where the disruption experiment for DBM control was conducted (Nemoto et al. 1980). *Sl*NPV also shows potential in the control *S. litura* (Nemoto and Okada 1990). These are alternative pest control measures for *S. litura*.

However, we do not have nonchemical control methods for aphids and common cabbageworm. Ground-crawling predators such as lycosid spiders played an important role as a biotic mortality agent of immature stages of DBM. Pest management in crucifers requires the nonchemical control method and application of selective insecticides. The chitin synthesis inhibitors, which are selective, were highly effective on caterpillars like common cabbageworm.

Pheromone and chitin synthesis inhibitors are regarded to be the best means of pest management in crucifers.

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Control of Diamondback Moth Using Synthetic Sex Pheromones

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Abstract

The sex pheromone of diamondback moth, *Plutella xylostella* (L.), consists of two compounds, (Z)-11 hexadecenal and (Z)-11 hexadecenyl acetate. Mass trapping of male moths by this pheromone in the field failed because of very limited active space of this pheromone. We then attempted communication disruption by using a commercial product 'Konaga-con' which is a polyethylene rope containing pheromone mixture, 25 g/100 m. The pheromone evaporates through the surface of polyethylene constantly for 3-4 months. Most results of open field experiments in Japan between 1984 and 1989 are successful. Maintenance at uniform high concentration of sex pheromone in the air throughout the season is critical. Major problems in achieving this are as follows: small fields, fields on steep slopes and strong winds. The results of green-house, vinyl house or plastic tunnel experiments were generally good. Under airtight conditions, almost complete inhibition in diamondback moth reproduction could be obtained. In ventilated areas the insect control was poor. Sex pheromone has no insecticidal action. Therefore, if the insect population density increases during the application of sex pheromone, use of insecticides may be necessary. Use of sex pheromone can reduce the need for insecticide application to less than a half.

Introduction

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), is a notorious pest of cruciferous vegetables in many parts of the world. In Japan, since 1965 it has become the most serious pest of cabbage, Japanese radish (daikon) and Chinese cabbage. To control DBM, large amounts of insecticides have been applied. However DBM has recently developed resistance to many insecticides and only a few remain that can control this insect. We have therefore been trying to use synthetic sex pheromones since the early 1980s to control this pest.

Sex pheromones to control harmful insects have been studied for the past 15 years. For some insects, application methods had already been established and are being used commercially. However newer techniques are being developed constantly that are easier to apply and less costly and more effective than insecticides.

The structure of the female sex pheromone of DBM has been determined by Tamaki et al. (1977). It consists of two compounds: (Z)-11 hexadecenal and (Z)-11 hexadecenyl acetate. First we tried a mass trapping technique to catch entire male moth population in the field, but this did not work. This is because of a very narrow active space of this pheromone. We then used the communication disruption. The results of that test are described here.

Materials and Methods

Pheromone: We used Konaga-con (Shin-Etsu Chemical Co., Ltd.), which is a polyethylene tube with an aluminum wire and containing 1:1 mixture of two sex pheromone components, together at 25 g AI/100 m tube. The pheromone evaporates through the surface of the polyethylene constantly for about 3-4 months.

Application methods of Konaga-con in open field: Tie the end of the rope at the top of a stake, which should be 40-50 cm high. Stretch the rope tightly between stakes placed at approximately 10-m intervals. Keep the rope tight stretching it along rows at intervals of 8-9 m. This amounts to the application rate of 100 m/0.1 ha.

Communication disruption effect: To examine the effect of communication disruption, we used the female sex pheromone trap. If the male DBM were confused with the pheromone from the tube, they could not approach the trap, even when the population density of mature moths was at a high level.

Mating inhibition effect: We used the tethered female method to examine the effect of mating inhibition. We tied the forewing of virgin female moths with fine thread and released it on the crop before normal mating time. We collected the females the next morning and checked the hatching of eggs. By comparing the mating ratio of pheromone-treated with the nontreated field, we can calculate the mating inhibition ratio.

Results

Effects of Konaga-con in open vegetable fields: In 1985, we applied Konaga-con on 5 ha of open radish field. Initial density of moths was low, and remained low throughout the experiment (Fig. 1). Attraction of the male moth to the monitoring trap was evidently inhibited, and the population level was lower than in the nontreated field without chemicals. The good control of DBM on summer cabbage cultivation in a high altitude field is shown in Fig. 2. In this case, the population density of moth was high. The pheromone treatment was done on a 10-ha field where wind velocity was less. The control of DBM at this higher altitude sloped field was not satisfactory. Results of an experiment on a windy field located on a cliff are given in Fig. 3. The DBM population density on the treated field was not suppressed even when the attractive inhibition was evident.

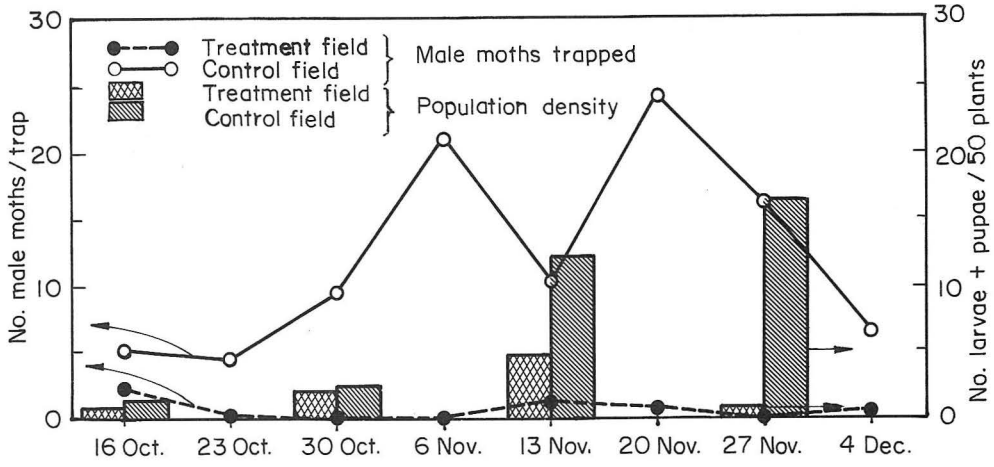

Fig. 1. Effect of Konaga-con in open radish field, Miura. 5.4 ha, 1985.

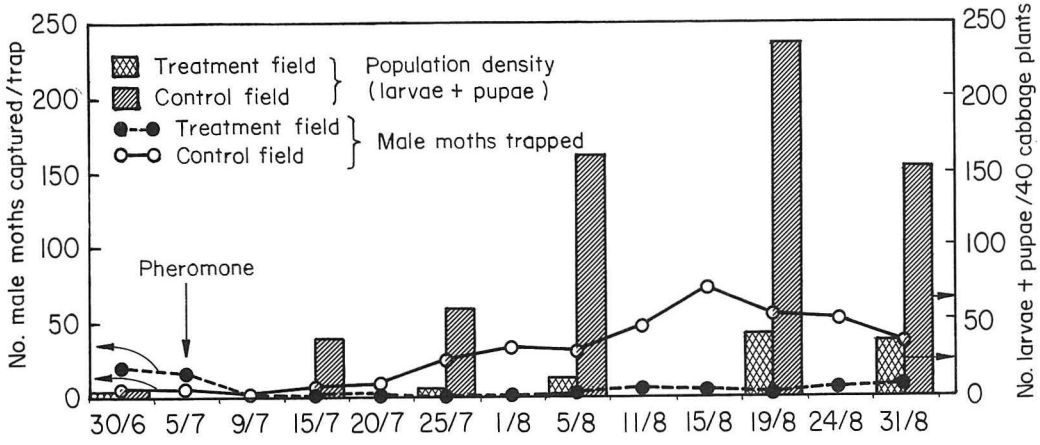

Fig. 2. Effect of Konaga-con in open cabbage field, Gumma. 10 ha, 1988.

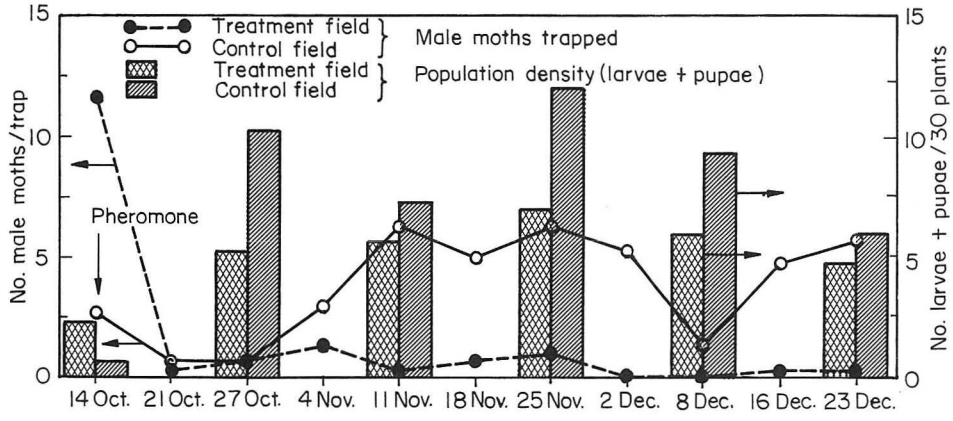

Fig. 3. Effect of Konaga-con in open cabbage field, Miura. 7.0 ha, 1987.

The results of the open field experiment in various parts of Japan from 1985 to 1988 are given in Table 1. The problems of pheromone application in getting consistent DBM control are shown by the results. For example, field No. 2 was a windy field on a cliff. It had high DBM population (Fig. 3); No. 4 showed good effects until mid May when the population of surrounding fields increased. Number 5 was a very complicated situation, with cabbage in an ornamental field and Japanese radish under plastic tunnel. The Konaga-con was therefore applied mainly on the cabbage and radish fields, but the radishes were harvested early, and the farmer removed the pheromone tube from the field. Number 9 was a small field that showed good control at the center, but inferior near the edge (Fig. 4). The results of the experiment at Gumma Prefecture in 1988 are shown in Fig. 5. Two chemical applications in addition to pheromone ensured DBM control.

Application methods of Konaga-con under structure: The results of greenhouse, vinyl house or plastic tunnel experiments are given in Table 2. To determine why some of the results were good and others poor, we tested the mating inhibition effect by modifying ventilation in plastic houses or methods of setting the pheromone tubes. (Table 3). If the house was under airtight conditions, an almost complete inhibition effect could be obtained. However, when the house was ventilated, the effect was reduced. The pheromone tube was more effective when

Table 1. Control effect of communication disruption method in open field in various parts of Japan.

Exp. No.[a]	Period of experiment	Attractive inhibition	Mating inhibition	Population density reduction
1	10.16-12.20	93.9%	100.0% (11.20)	95.5%(11.27)
2	9.30-12.23	89.3	74.6 (11.17)	49.5 (11.25)
3	10.13-12.22	88.7	88.4 (11.28)	85.7 (11.10)
4	3.27-5.28	94.7	97.1 (5.1)	51.5 (5.28)
5	3.26-6.12	83.0	56.8 (5.13)	9.7 (6.12)
6	9.30-12.23	92.4	90.6 (11.7)	86.1 (11.11)
7	7.1-8.27	95.2	100.0 (7.23)	94.2 (7.23)
8	7.5-8.19	90.3	100.0 (8.16)	82.4 (8.19)
9	7.5-8.19	87.6	100.0 (8.16)	99.6 (8.19)
9[b]			57.2 (8.16)	47.0 (7.25)

[a] 1. Miura, Kanagawa 1985 5.4 ha, radish field
2. Miura, Kanagawa 1987 2.7 cabbage field
3. Miura, Kanagawa 1988 3.2 cabbage
4. Yokohama, Kanagawa 1986 3.1 cabbage
5. Yokohama, Kanagawa 1987 7.0 cabbage
6. Choshi, Chiba 1988 14.4 cabbage
7. Tsumagoi, Gumma 1987 9.8 cabbage
8. Tsumagoi, Gumma 1988 10.0 cabbage
9. Tsumagoi, Gumma 1988 0.8 cabbage

[b] 9. Same as above field but data were obtained from edge of field.

Source: Ohbayashi et al. 1989.

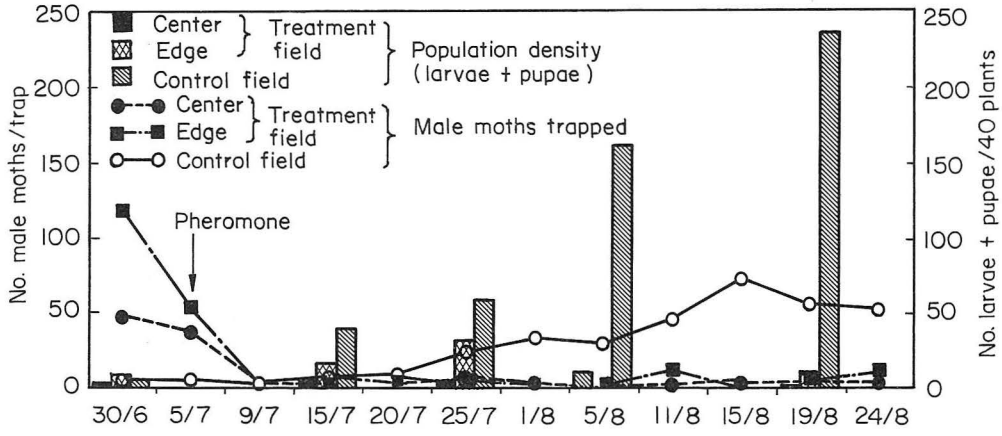

Fig. 4. Effect of Konaga-con in open cabbage field, Gumma 0.8 ha.

placed on the ceiling than on the top of cabbages. These results indicate that the active ingredients of sex pheromone must be kept high so that the pheromone will uniformly cover the crop where the mating takes place. It is therefore necessary to keep the house tightly shut in the evening and night when the DBM are mating. Comparison of concentrations of pheromone indicated that 200 m rope/0.1 ha stabilized the effect, and the scattered placement of pheromone tubes was better than concentrated application. Kawana and Shimizu (1990) have also shown that 400 m rope was superior to 100-200 m rope/0.1 ha.

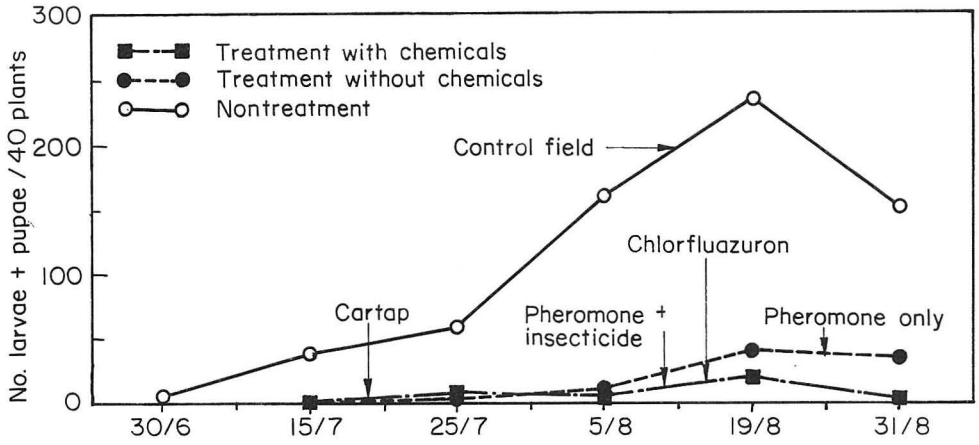

Fig. 5. Effect of Konaga-con with insecticides in open cabbage field, Gumma. 10 ha. 1988.

Table 2. Control effect of communication disruption method in greenhouse, vinyl house or plastic tunnel.

Exp. No.[a]	Period of experiment	Mating inhibition[b] (%)	Population density[b] reduction (%)
1	10.12-12.10	100.0 (10.16)	90.3 (11.27)
2	3.23-5.12	48.6 (5.12)	84.2 (4.30)
3	9.27-11.22	83.3 (11.22)	43.0 (11.22)
3	9.27-11.22	38.2 (11.22)	39.5 (11.22)
4	12.7-3.13	100.0 (3.13)	69.1 (3.13)
5	10.28-1.7	100.0 (12.15)	42.1 (12.4)
6	10.28-1.7	93.6 (12.15)	43.9 (12.4)

[a]1. Miura, Kanagawa 1984 greenhouse 325m/0.1ha Cabbage
2. Miura, Kanagawa 1987 greenhouse 300m/0.1ha Radish
3. Miura, Kanagawa 1989 vinyl-house 100m/0.1ha Cabbage
3. Miura, Kanagawa vinyl-house 300m/0.1ha
4. Choshi, Chiba 1985 plastic tunnel 50m/0.1ha Radish
5. Choshi, Chiba 1987 plastic tunnel 50m/0.1ha Radish
6. Choshi, Chiba 1987 plastic tunnel 100m/0.1ha Radish

[b]Figures in parentheses are observation dates.
Source: Ohbayashi et al. 1989.

Table 3. Influence of ventilation and position of placement of sex pheromone (25 g/0.1 ha) on the mating inhibition in plastic house.

Ventilation method	Setting place of pheromone	Mating inhibition rate (%)
Bottom	on the ceiling	11.8
	over head of cabbage	7.1
Shoulder	on the ceiling	72.9
	over head of cabbage	40.9
No ventilation (airtight)	on the ceiling	100.0
	over head of cabbage	78.8

Ohbayashi et al. 1989.

Discussion

Most female lepidopterous insects emit the sex pheromone to lure males of the same species. Males find the females by following the smell of the sex pheromone and then they mate. When the field is covered with sex pheromone, the communication for mating is confused. The mode of action of the pheromone is such as to affect the mating of DBM resulting in a reduction of the population. The communication disruption technique using DBM sex pheromone (Konaga-con) is quite useful in reducing the population density when applied on a field (> 3 ha). The results show that it is most important to keep the sex pheromone ingredient in the air constantly. This is difficult, of course, in small fields, steeply sloping fields, fields on a cliff, or fields in strong wind areas. It is also necessary to apply the pheromone at the beginning of emergence of moths, and to avoid applying on windy and sloping fields. Besides being species specific, the sex pheromone has no insecticidal function, so it is necessary to apply an insecticide if the population density of DBM itself or the other insect increases during the application of sex pheromone. The use of sex pheromone can usually reduce the need of chemical application to less than one half. We are continuing our research on the application of Konaga-con to small open fields and to greenhouse, vinyl house and plastic tunnels.

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12

Disruption Effect of the Synthetic Sex Pheromone and its Analogues on Diamondback Moth

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Abstract

Five mg and 50 mg of sex pheromone (Z-11-16:Ald, Z-11-16:Ac and Z-11-16:OH), 5 mg and 10 mg of Z-9-14:Ac and 1 mg and 10 mg of Z-11-16:OH were tested to show the disruption effect on diamondback moth, *Plutella xylostella* (L.), in a field. When the distance between the sticky trap and tested chemical was zero or 2.8 m, the chemicals did not show the disruption effect. However, when the distance increased to 6.3 or 8.4 m, all tested chemicals,except 1 mg of Z-11-16:OH, showed the disruption effect. In control traps more males were attracted to the long distance (6.3 and 8.4 m) than to the short one (0 and 2.8 m). The data obtained from this observation suggest that the active space of DBM in the field is possibly between 0 and 6.3 m.

Introduction

Diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera:Yponomeutidae), is one of the serious pests of cruciferous vegetables in Taiwan. Its sex pheromone has been identified as a mixture of (Z)-11-hexadecenal (Z-11-16:Ald) and (Z)-11 hexadecenyl acetate (Z-11-16:Ac) (Tamaki et al. 1977). (Z)-11-hexadecen-1-ol (Z-11-16:OH) and (Z)-9-tetradecenyl acetate (Z-9-14:Ac) were thought to be parts of the DBM's pheromone system (Ando et al. 1979; Koshihara and Yamada 1981; Chisholm et al. 1983). The ratio and dosages of the components of DBM pheromone were important factors that affect the pheromone activity (Chow et al. 1977; Koshihara et al. 1978; Koshihara and Yamada 1980; Chisholm et al. 1979; Lin et al. 1982). The optimal dosage of the sex pheromone of DBM in the field was 50-100 μg of the mixture Z-11-16:Ald, Z-11-16:Ac and Z-11-16:OH in the ratio of 5:5:0.1 in rubber septa (Koshihara and Yamada 1980); in Canada the optimal dosage was Z-11-16:Ald (70 μg), Z-11-16:Ac (30 μg), Z-11-16:OH (1 μg) and Z-9-14:Ac (0.01 μg) (Chisholm et al. 1983).

The use of high dosages of synthetic compounds similar or related to the true sex pheromone to confuse the communication between insects to prevent mating is called the disruption method (Hirano 1979; Nakasuji 1979). This control method has been successfully conducted on gypsy moth (*Lymantria dispar*), and *Spodoptera litura* in the field (Cameron et al. 1975; Shorey et al. 1974; Nakasuji 1979; Hirano 1979). However, in DBM, only mating inhibition has been demonstrated in the laboratory by its main components or minor Z-11-16:OH (Fujiyoshi et al. 1979; Lin and Chow 1982). In the present study, the disruption effects of synthetic sex pheromone on DBM were evaluated in the field in Hsin Chu County in Taiwan.

Materials and Methods

Two separate experiments were carried out in a cabbage field. Five mg and 50 mg of sex pheromone (Z-11-16:Ald, Z-11-16:Ac and Z-11-16:OH in the ratio of 5:5:0.1), 5 mg and 10 mg of Z-9-14:Ac, and 1 mg and 10 mg of Z-11-16:OH were tested as disruption chemicals.

The sticky traps baited with 50 μg of the sex pheromone in the ratio 5:5:0.1 in polyethylene microtubes were used as a monitoring trap (Lin et al. 1982). The height of the trap was set the same height as the cabbages. Field design for location and distance of sticky trap and tested chemicals is shown in Fig. 1.

The number of male moths attracted was recorded every 3 or 4 days. The data obtained were analyzed with analysis of variance and Duncan's multiple range test to show the disruption effect of the tested chemicals on DBM.

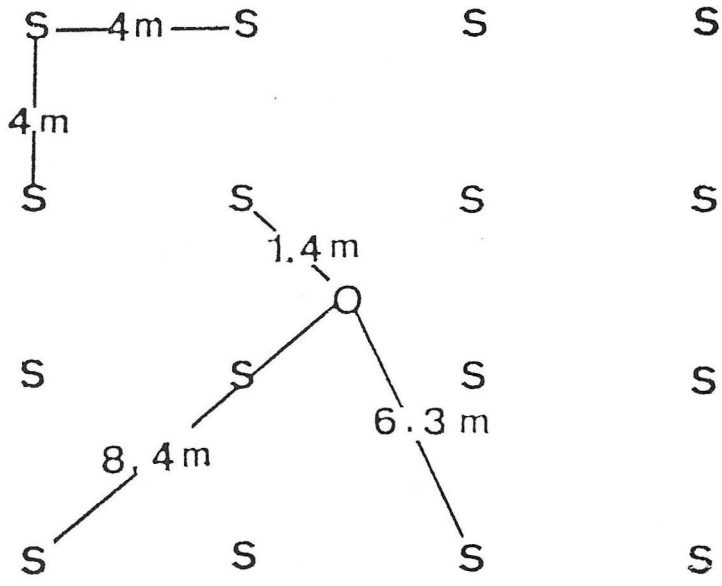

Fig. 1. Field design for location and distance of sticky trap and tested chemicals (S: sticky trap with 50 μg of sex pheromone, O: original disruption point with 50 mg of sex pheromone, and other tested chemicals or no chemicals.

Results

The results are presented in Table 1. The average number of attracted males per trap in the control traps was not significantly different from that in other treatments when the distance between sticky trap and tested chemical was zero or 2.8 m. When the distance between sticky trap and chemical increased to 6.3 m or 8.4 m, the results of the control and Z-11-16:OH (1 mg) traps were significantly different from those of the others. Therefore, 5 and 50 mg of sex pheromones, 5 and 10 mg of Z-9-14:Ac and 10 mg of Z-11-16:OH showed disruption effects, i.e. DBM males could not locate the female partners effectively. One mg of Z-11-16:OH did not show the disruption effect. On the other hand, except for 1 mg of Z-11-16:OH, the mean catch of control (10.8 males) was higher than other treatments (5.6-7.8 males). So a disruption effect did exist in these observations.

Discussion

Koshihara and Yamada (1980) used 0.01, 0.1, 1 and 10 mg of Z-11-16:Ald, Z-11-16:Ac and Z-11-16:OH (5:5:0.1) to test the attraction of DBM and found that 0.01-1 mg were optimal

dosages and few if any males were attracted by the 10 mg dosage. Our results confirmed these data that higher dosages (5-10 mg) of sex pheromone could show the disruption effect. Koshihara and Yamada (1980) also used the mixtures of Z-11-16:Ald and Z-11-16:OH in the ratio of 5:5 at the 0.01 mg level, to which Z-11-16:OH was added in different ratios (0, 0.01, 0.1, 1 and 10 μg), and found that 10 μg of Z-11-16:OH had the least effect in attracting the male moths. Our results indirectly support their data that the higher dosage (10 mg) of Z-11-16:OH has the best disruption effect (last column in Table 1). Chisholm et al. (1983) evaluated the attractiveness of Z-11-16:Ald (70 μg), Z-11-16:Ac (30 μg), Z-11-16:OH (1 μg) and Z-9-14:Ac (0.01 to 10 μg) and found that when higher dosage of Z-9-14:Ac was used, a lower number of male moths were attracted. Our findings also support these results.

The average number of males per trap in the control was not significantly different from other experimental treatments in the shorter distance (0 m). However, in longer ones (6.3 and 8.4 m), the control and 1 mg of Z-11-16:OH were significantly different. In the control, more males were attracted in the long distance (6.3 and 8.4 m) than in the short one (0 and 2.8 m). Therefore, it seemed that there was a disruption effect among monitoring traps in the short distance but little or no effect in the long one. In the middle range (2.8 m), the different effects of Z-9-14:AC and Z-11-16:OH were obtained in the experimental treatment but not in the control. The reason for this may be the different thresholds of the male for the female sex pheromone. Under the influence of disruptive chemicals, the males respond with a higher threshold. Therefore, the hypothesis that the active space of sex pheromone of DBM is 4-5 m as proposed by Ohbayashi et al. (1989) is reasonably true. The factors affecting the pheromone activity such as wind speed, temperature, population density, etc., varied widely under field conditions. The data obtained in this study suggest that the active space of sex pheromone of DBM in the field is greater than 1 m, possibly up to 6.3 m.

Table 1. Trapping of DBM males 3 or 4 days for different tested chemicals and different distances.

Treatment		No. males trapped per plot set at distance between tested chemicals and sticky trap of				
		0 m	2.8 m	6.3 m	8.4 m	mean
Sex Pheromone	5 mg	5.0 a	6.1 bc	8.2 b	8.4 b	6.9
Sex Pheromone	50 mg	3.3 a	5.9 bc	9.5 b	12.5 b	7.8
Z-9-14:Ac	5 mg	3.4 a	8.6 a	9.0 b	9.8 b	7.7
Z-9-14:Ac	10 mg	2.8 a	8.2 a	9.1 b	9.3 b	7.3
Z-11-16:OH	1 mg	4.9 a	7.4 ab	12.8 a	18.6 a	10.9
Z-11-16:OH	10 mg	3.0 a	4.9 c	7.3 b	7.3 b	5.6
Control		5.5 a	6.8 abc	12.5 a	18.7 a	10.8

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13

Evaluation of Communication Disruption Method Using Synthetic Sex Pheromone to Suppress Diamondback Moth Infestations

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Abstract

Field tests were conducted in 1987 and 1988 to evaluate the communication disruption method for the control of diamondback moth, *Plutella xylostella* (L.), on cabbage using a synthetic sex pheromone dispenser (SSPD) containing (Z)-11-hexadecenal: (Z)-11-hexadecenyl acetate (36:41). Fields for the SSPD setting were flat with strong winds in the Atsumi Peninsula and undulating mountainous area in Sitara, the northern district of Atsumi. The cabbage-growing season in Atsumi is from September to March, and in Sitara from June to October. The SSPD was set on the cabbage field when temperature was decreasing in Atsumi, and increasing in Sitara. The DBM adult population density in the field with SSPD treatment decreased by 92 to 97% in Atsumi and 95% in Sitara compared to that in the field without SSPD treatment. The mating rate in the SSPD-treated field in Atsumi was only 5.4 and 3.3% at 13 and 41 days after the SSPD setting, respectively, while that in untreated field was 50.9 and 30.4%. In Sitara, the former was only 0 and 5.3% at 20 and 63 days after the SSPD treatment, while the latter was 24.4 and 74.5%. In both of these SSPD-treated areas the larval population remained low. Total dose of the synthetic sex pheromone released from the dispenser was higher in summer than in winter. These results indicate that efficacy of DBM control by the communication disruption method using SSPD is not affected by meteorological or topographical conditions.

Introduction

Insecticide resistance of the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), caused by frequent field application of chemical insecticides has been a serious problem in the Atsumi Peninsula of Japan (Nishiwaki et al. 1988). We therefore tried to introduce the synthetic female sex pheromone as a DBM control agent.

Tamaki et al. (1977) reported (Z)-11-hexadecenal and (Z)-11-hexadecenyl acetate as the sex pheromone of the DBM, and showed that the male was attracted by 1:1 or 4:6 mixtures of these components. Fujiyosi et al. (1979) showed mating disruption of DBM using the 1:1 mixture in laboratory experiments. The purpose of our study was to evaluate synthetic sex pheromone to control DBM infestation in cabbage fields of the Atsumi Peninsula and Sitara.

Materials and Methods

Experimental fields

Cabbage fields in Atsumi and Sitara where the crop is cultivated successively were selected. These areas differed in cabbage transplanting time, temperatures during the growing

season and topography. Atsumi is situated in Atsumi Peninsula in the central district of the Japanese Archipelago. The cabbage field in Atsumi was a flat area near the sea with strong winds. Cabbages are transplanted in early autumn and harvested in late winter to spring. The temperature decreases as the season progresses. The Sitara cabbage field is in a mountainous region about 100 km north of Atsumi. The cabbage field is about 1000 m above sea level. Cabbage is cultivated in a sloping field, and are transplanted in early summer and harvested in mid summer to autumn. Here the temperature increases as the season progresses.

Synthetic sex pheromone dispenser

The synthetic sex pheromone dispenser (SSPD) used in the present study contained 36:41 mixture of (Z)-11-hexadecenal and (Z)-11-hexadecenyl acetate sealed in polyethylene tubes (Shin 'Etsu Chem. Co. Ltd). Ten thousand meters of SSPD (250 g AI/ha) was suspended over 0.1 ha cabbage field. SSPD was set on 17 September 1987 in Atsumi, and on 8 June 1988 in Sitara, 30-40 cm above the ground at 10-m intervals after the cabbage transplanting, and was maintained until cabbage harvest time.

Monitoring sex pheromone trap for adult population estimation

Sticky type of monitoring sex pheromone traps (Zoecon Corp.) were set 1 m above the ground in order to evaluate the effect on communication disruption. Each trap was baited with a rubber septum with a mixture of synthetic (Z)-11-hexadecenal. (Z)-11-hexadecenyl acetate and (Z)-11-hexadecenol (5:5:0.1) at a total dose of 0.1 mg (Takeda Chem. Ind. Ltd.). The pheromone traps were set in six plots in the SSPD-treated field and in two plots in the untreated field 7 days before SSPD treatment. Each trap was checked every week for 12 weeks from 17 September to 15 December 1987 in Atsumi, and 13 times in Sitara from 8 June to 31 August 1988.

Mating rate estimation

Estimation of mating rate was done using tethered female moths. In Atsumi, the estimation was conducted 13 days and 41 days after the SSPD setting. It employed 10 tethered female moths in six plots in the SSPD-treated field, and 30 tethered female moths in two plots in the untreated field. In Sitara, a similar procedure was followed at 20 and 60 days after the SSPD setting. Twenty tethered female moths were employed in three plots in the SSPD-treated field and 30 tethered female moths in two plots in the untreated field.

The procedure for mating rate estimation is as follows: Freshly emerged virgin female moths were collected. The forewing of each moth was tied with fine nylon thread 20 cm in length in the plastic tube (1 cm in diameter and 5 cm in depth). In the evening, the tethered female moths in the plastic tube is put randomly on the cabbage in the experimental field. They were recovered the next morning and transferred to the laboratory, and the eggs laid in the plastic tube were examined for hatchability.

Larval population density of the DBM

Larval population in the cabbage fields was counted once a week for 11 weeks from 17 September to 3 December 1987 in Atsumi, and for 12 weeks from 8 June to 31 August 1988 in Sitara. The number of larvae/20 cabbages at each plot were counted in six plots in the SSPD-treated field and at two plots in the untreated field.

Estimation of the evaporated dose of the synthetic sex pheromone

The dose of the synthetic sex pheromone that evaporated was estimated at Aichi-ken Agricultural Research Center located at Nagakute and Sitara. SSPD of 100 m (250 mg AI/m)

long was set 1 m from the ground in sunshine and shade. The weight was measured 12 times once a week from 18 September to 18 December 1987 at Nagakute and 11 times once a week from 22 June to 31 August 1988 at Nagakute and Sitara under only sunshine conditions.

Results and Discussion

1. Effect of SSPD at Atsumi

Occurrence and abundance of DBM adult population. Occurrence and abundance of captured DBM male adults by the monitoring sex pheromone trap between the SSPD-treated cabbage field and the untreated field in Atsumi are shown in Fig. 1. The male adult population density in the field with SSPD treatment decreased to 92 to 97% of that in the field without SSPD treatment during late October to mid November when adult population density peaks. During the experimental period, the total number of adult moths captured by the monitoring sex pheromone trap was 4538 adults/trap in the check field, and 213 adults/trap in the SSPD-treated field. These results showed that communication disruption of adult DBM certainly continued throughout SSPD treatment in the cabbage field.

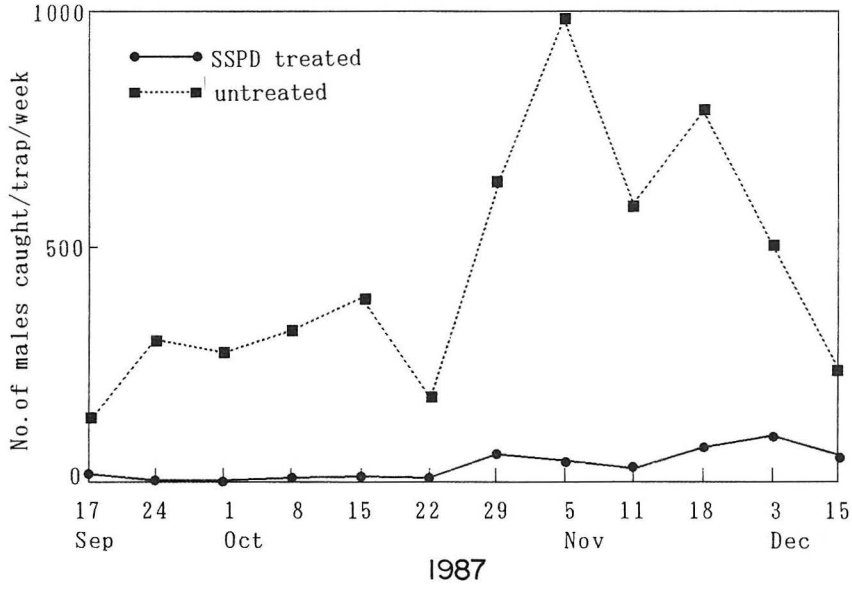

Fig. 1. Occurrence and abundance of captured DBM male adults by the monitoring sex pheromone trap between SSPD-treated and untreated cabbage fields in Atsumi.

Mating rate. The mating rates (number of females mated/number of females recovered alive) of the tethered female moths in the SSPD-treated field were 5.4% (3/56) and 3.3% (2/60) at 13 and 41 days after the SSPD setting, respectively, while those in the untreated field were 50.9% (27/53) and 30.4% (17/56), respectively (Table 1). It seems that these low levels of mating in SSPD-treated fields are the result of mating inhibition caused by the pheromone dispenser treatment.

Occurrence and abundance of DBM larval population. Suppression effects of SSPD treatment against DBM larval populations in the cabbage field in Atsumi are shown in Fig. 2.

Table 1. Mating rates of the tethered DBM female adults in SSPD-treated and control field.

SSPD	Atsumi 1987 Mating rate (%) after		Sitara 1988 Mating rate (%) after	
	13 days	41 days	20 days	63 days
Treated	5.4	3.3	0	5.3
Untreated	50.9	30.4	24.4	74.5

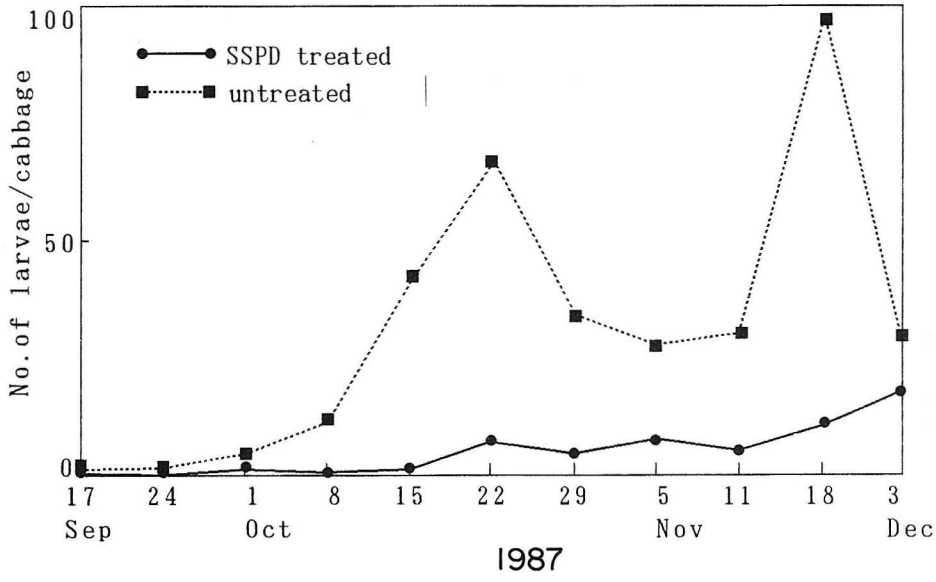

Fig. 2. Suppression effect of SSPD against DBM larval population in cabbage field in Atsumi.

The peak larval population was observed in late October and mid November when 66.5 and 98.1 larvae were found per cabbage plant in the SSPD-treated and check field, respectively. This decrease of DBM larval population resulted from the communication disruption, and mating inhibition of adult moths continued at an extremely low level throughout the period of the SSPD treatment.

Evaporation of the synthetic sex pheromone in SSPD. The dose of the synthetic sex pheromone that evaporated from SSPD in two different circumstances, sunshine and shade, were 2.91 and 2.77 mg/m/day, respectively, just after SSPD setting in mid September, and 0.63 and 0.45 mg/m/day, respectively, at 80 days after the SSPD setting (early December). These results indicated that the release rate of the synthetic sex pheromone from SSPD decreased with decreasing temperature. Total dose of the synthetic sex pheromone evaporated from SSPD was 107.5 mg/m under sunshine conditions, showing that 43.0% of active ingredient was evaporated during 91 days from September to December. There was no difference between sunshine and shade conditions in the dose evaporated.

2. Effect of SSPD at Sitara

Occurrence and abundance of DBM adult population. Occurrence and abundance of captured DBM male adults by the monitoring sex pheromone trap between SSPD treated cabbage

field and untreated field in Sitara are shown in Fig. 3. The male adult population density in the field with SSPD treatment decreased to 95 to 96% of that in the field without SSPD treatment, during mid July to early August when adult population density peaked. During the experimental period, the total number of adult moths captured by the sex pheromone monitoring trap was 789 adults/trap in the check field, and 72 adults/trap in the SSPD-treated field. These results showed that communication disruption of adult DBM certainly continued throughout SSPD treatment in the cabbage field.

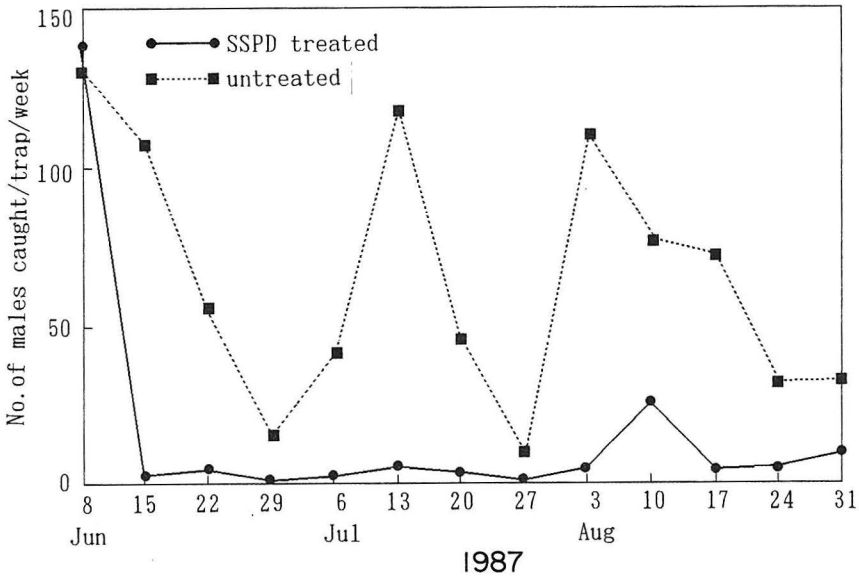

Fig. 3. Occurrence and abundance of captured DBM male adults by the sex pheromone monitoring trap between SSPD-treated and untreated cabbage field in Sitara.

Mating rate. The mating rates of the tethered female moths in the SSPD-treated field were 0% (0/46) and 5.3% (3/57) at 20 and 60 days after the SSPD setting, respectively, whereas those in the untreated field were 24.4% (10/41) and 74.5% (41/55), respectively (Table 1). These data showed that mating inhibition caused by the SSPD treatment was found also in the mountainous region.

Occurrence and abundance of DBM larval population. Suppression effect of SSPD treatment against DBM larval populations at two different cabbage fields in Sitara is shown in Fig. 4. In the first experiment the field where cabbages were harvested in late July (top figure), in the check field the peak larval population was found in late June and mid July when 8 and 33 larvae/cabbage were found. In the second experiment where cabbages were harvested in late August (bottom figure), the peak of larval population was observed in late July and early August. In this experiment 24.9 and 52.7 larvae were found in a cabbage in the check field. In both cabbage fields, the larval population remained at a negligible level throughout SSPD treatment.

Evaporation of the synthetic sex pheromone in SSPD. The dose of the synthetic sex pheromone lost in Sitara and Nagakute was 1.43 and 2.63 mg/m/day, respectively, immediately after SSPD setting in late June. In mid July the peaks of the evaporated dose (2.5 and 3.14 mg/m/day) were seen in Sitara and Nagakute. The release rate of the synthetic sex pheromone

then decreased gradually, reaching a minimum of 1.07 mg/m/day in Sitara, and 1 mg/m/day in Nagakute at 77 days after SSPD setting (late August). Total dose of the synthetic sex pheromone evaporated was 115.1 mg/m, accounting for 46% of total active ingredient in Sitara, and 151.7 mg/m or 60.7% in Nagakute. It seems likely that higher evaporation of the synthetic sex pheromone in Nagakute is due to the higher average temperature (+3.1°C) as compared with that in Sitara.

These results indicate that efficacy of communication disruption with synthetic sex pheromone dispenser against DBM was not affected by meteorological or topographical conditions.

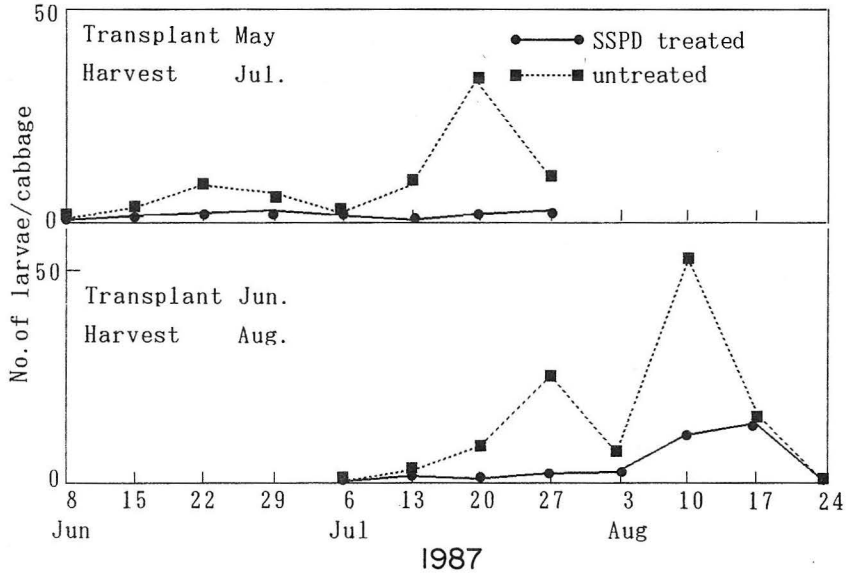

Fig. 4. Suppression effect of SSPD against DBM larval population in cabbage cultivation area in Sitara.

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Control of the Beet Armyworm in Open Fields with Sex Pheromone

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Abstract

The feasibility of synthetic sex pheromone as a communication disruption agent for the control of the beet armyworm, *Spodoptera exigua* (Hübner), was tested using a 7:3 mixture of (*Z,E*)-9,12-tetradecadienyl acetate and (*Z*)-9-tetradecen-1-ol. When dispersed into a 155-ha field, attraction of male moths to sex pheromone traps was completely inhibited and densities of egg masses and young larvae were reduced to 6 and 1%, respectively, relative to those in an untreated field about 9 km away. Follow-up studies enabled us to estimate that rate of mating inhibition in the treated field was about 97%. Pheromone treatment shows potential for reducing the population density in open fields. Evaluation techniques of the effects of sex pheromone treatment will be discussed.

Introduction

The beet armyworm (BAW), *Spodoptera exigua* (Hübner), (Lepidoptera: Pyralidae), is a serious pest of cabbage in Southeast Asia, especially in Thailand. This insect has also attacked Welsh onion (*Allium fistulosum* L.) fields in Kochi and Kagoshima Prefectures in Japan since the early 1980s (Horikiri 1986; Takai 1988a, 1989). The effectiveness of most of the insecticides used (including methomyl and EPN) has declined. Insecticides were certainly effective in the early 1980s (Takai 1988b), but this species appears to have the potential to rapidly acquire resistance (Takai 1988b; Meinke and Ware 1978). The development of a new technique other than insecticide spraying was necessary to control this insect. This prompted us to study the feasibility of disrupting communication using synthetic sex pheromone.

Brady and Ganyard (1972) identified one of the sex pheromone components of BAW as (*Z,E*)-9,12-tetradecadienyl acetate (Z9E12-14:Ac). Mitchell and Doolittle (1976), however, showed that this component had no attractant activity by itself. Tumlinson et al. (1981) reinvestigated the sex pheromone components, identified 11 compounds from virgin female secretions, and revealed that (*Z*)-9-tetradecen-1-ol (Z9-14:OH) was also an essential component for male attraction. Mitchell et al. (1983) reported an effective formulation: a mixture of 0.1 mg of Z9E12-14:Ac and 0.01 mg of Z9-14:OH on a rubber septum. In Japan and Taiwan, this formulation was demonstrated to be effective for male attraction (Wakamura 1987; Cheng et al. 1985), and to be useful for monitoring seasonal occurrence.

Communication Disruption in Open Fields

Experiments were conducted in two areas, Nii and Kitahara, Tosa City, Kochi Prefecture, Japan, in 1987. The treated area (Nii) was about 155 ha, of which Welsh onion plots comprised

about 24 ha (Wakamura et al. 1989, 1990b). This area was regarded as isolated from other agricultural areas; the west side of this area faces forest and the northwest side faces a river bordered by forest. To the south of the field is a small residential area bordered by the ocean. The untreated area (Kitahara) was 9 km from the treated one.

Since no information was available on the dispersal distance of adult BAW, we assumed that it has flight potential equalling that of adult *S. litura*. *S. litura* males are able to fly more than 5 km during one night (Oyama and Wakamura 1976; Wakamura et al. 1990a). Females have equal flight potential (Noda and Kamano 1988). Sex pheromone permeation of a field certainly inhibited mating behavior, but often resulted only in a slight reduction of larval population, possibly because of immigration of fertilized females from outside the treated area (Oyama et al. 1978; Kitamura et al. 1985; Kitamura and Kobayashi 1985). We attempted to permeate as large an area as possible with the largest possible amount of synthetic sex pheromone.

The dispensers of synthetic sex pheromone were supplied by Shin'Etsu Chem. Co. Ltd.; a sealed polyethylene tube 20 cm long and containing 80 mg of a 7:3 mixture of Z9E12-14:Ac and Z9-14:OH and an aluminum wire. Twenty-four thousand dispensers were set evenly in the Welsh onion fields at the rate of 1000 dispensers/ha. In other parts of the treated area (about 130 ha) such as rice fields, greenhouses, orchards, home gardens and forests, about 42,000 dispensers were set at the rate of 320 dispensers/ha. In the open Welsh onion or rice fields, each release point had three dispensers attached to the top of a 60-cm plastic stick with vinyl adhesive tape. Trees and greenhouses had dispensers directly attached, at 1-1.5 m above the ground. The total number of dispensers was 66,000, and the total amount of sex pheromone used was about 5.3 kg.

In 1986, a large peak of trap catches of adult *S. exigua* was observed in September in sex pheromone and light traps. This peak was preceded by an increase in severe crop damage by larvae (Takai 1988a). In the present experiment, sex pheromone dispensers were set on 16-17 July 1987 to investigate the effect on the larval population from late August to early September; it was removed on 17 and 18 September to examine whether the population density would increase after the removal.

Effects of communication disruption

Water-pan type of sex pheromone traps (30 × 24 × 15 cm, Takeda Chem. Ind. Ltd.) were set 1 m above the ground at four locations in the treated area, and at two locations in the untreated area to evaluate the effect of communication disruption. Each trap was baited with a rubber septum impregnated with a 7:3 mixture of Z9E12-14:Ac and Z914:0H (Wakamura 1987). Each trap in the treated area was accompanied by an empty trap 10 m away to offset chance male catches (i.e. catches not by attraction). A light trap (lamp: FL-6) was set at the center of the treated area. Each trap was checked daily and captured BAW moths were stored in 70% ethanol. Females were dissected to investigate the spermatophore in the bursa copulatrix.

Two-day-old females were tethered and placed on tops of sticks arranged in the onion fields in treated and untreated areas as in Oyama (1974). On the evening of 27 August, 20 females were tethered in each of the two plots in the treated area, and 25 females in the untreated area. They were recovered the next morning and investigated for spermatophore.

BAW adults were captured with a light trap throughout the treatment period (Fig. 1). This indicates that adults were in the treated area throughout the experimental period. Conversely, mean trap catch of sex pheromone traps was as low as that of empty traps during the treatment period. These results showed that the effect of communication disruption certainly continued throughout the period.

The mating rate of the females caught with the light trap increased during the treatment period (Table 1): 40-60% in late July and early August, 70-80% in mid and late August, and 70-90% in early and mid September. After removal of the dispensers, the mating rate exceeded 90%. The mating rates of the tethered females were 0% [0/18 = (no. of females mated)/(no.

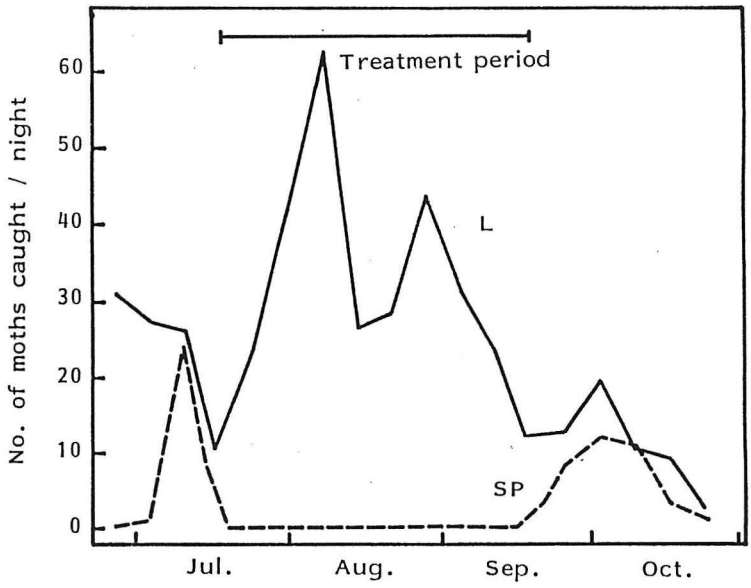

Fig. 1. Catches of BAW with sex pheromone traps (SP) and light trap (L) in the area treated with synthetic sex pheromone (1987, Nii).

Table 1. Catches of adult BAW with light trap in the treated area with synthetic sex pheromone (1987, Nii).

Date	No. of individuals caught				Mating ratio[b] (%)	Sex ratio[c] (%)
	Female		Male	Unknown[a]		
	Mated	Virgin				
7/24-7/30	16	24	134	9	40	23
7/31-8/6	59	43	97	8	58	51
8/7-8/13	28	7	79	2	80	69
8/14-8/20	21	7	77	2	75	27
8/21-8/27	35	15	33	2	70	60
8/29-9/3	93	36	121	7	72	52
9/4-9/10	35	5	62	3	88	39
9/11-9/17	27	11	47	6	71	45
	------removal of pheromone dispenser------					
9/18-9/24	42	4	31	5	91	60
9/25-10/1	58	0	45	9	100	56

[a]destruction of abdomen. [b](no. of females mated)/(no. of females caught) × 100. [c](no. of females caught)/(no. of females) + (no. of males) × 100.

of females recovered alive)] and 17% (3/18) in the treated area, and 92% (23/25) in the untreated area. Therefore, some females were thought to be able to mate even in the treated area.

Effects on density of BAW eggs and larvae

Onions were planted on about 1 m wide ridges in the plots (0.05-0.1 ha) which were scattered in the experimental areas. Larval field density was surveyed in every 5 or 6 plots in the central and marginal parts of the treated area, and in the untreated area once a week from 3 weeks

before the placement of dispensers (26 June) to 6 weeks after removal (30 October). Plot and ridge were arbitrarily selected where onion plants were 30-60 cm high.

Surveys were conducted on all of the 400 to 500 hills on 10 m of ridge of each plot. Most larvae were inside hollow leaves. Damaged leaves were collected and dissected to recognize the instar and to count the number of larvae. When the density became higher, fewer hills were sampled to save time and labor. Farmers sprayed insecticides such as methomyl, EPN, permethrin and fenvalerate-dimethoate against BAW, both in the treated and untreated areas, independently from the experiment. However, these insecticides were ineffective (Takai 1988b) and thus considered to have no effect on population density.

The mean densities of egg masses, 1st and 2nd instar larvae, and 4th and 5th instar larvae are shown in Fig. 2. In the treated area, the egg mass density was less than 0.5/100 hills,

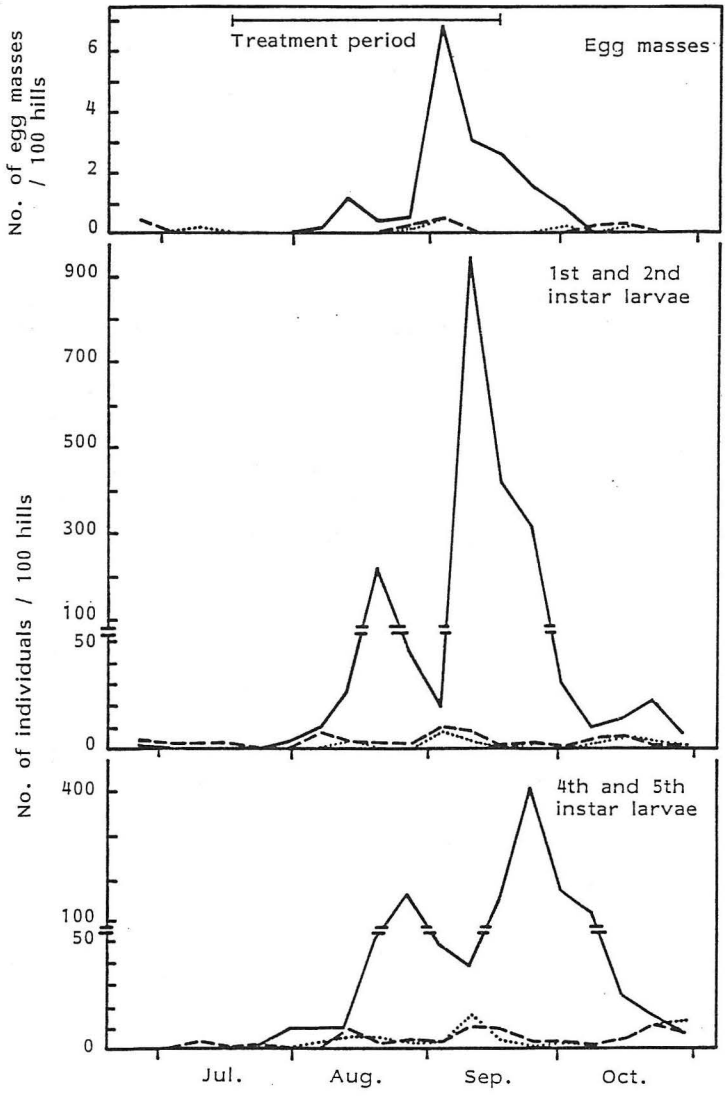

Fig. 2. Population densities of BAW egg masses and larvae in areas treated and not treated with synthetic sex pheromone in 1987. Broken and dotted lines indicate the densities in two survey areas. Solid line indicates densities in the untreated area.

both in the middle and peripheral plots throughout the period. Conversely, in the untreated area, mean egg mass density peaked twice. These peaks were followed by peaks of the 1st and 2nd instar larvae both in treated and untreated areas. They appeared 1 week after those of egg masses. In the treated area, the maximal density was about 10 individuals/100 hills compared with more than 900 individuals/100 hills in untreated areas. Egg mass density was considered to have been reduced in the treated area. However, all of the egg masses collected in both treated and untreated areas were observed to hatch normally. This suggests that hatchability was not reduced in the treated area. It is therefore apparent that the chance for the females to mate was reduced by the large amount of sex pheromone dispersed into the test area, which resulted in a decrease of density of egg masses and 4th and 5th instar larvae.

Communication disruption experiments were successful against several fruit tree and tea pests in Japan (Ohtaishi 1986; Furuno 1986; Sato 1986). Subsequently, some pest control agents have been commercially available. However, communication disruption experiments which aimed to control noctuid species have been reported for *S. litura* (Kitamura et al. 1985; Oyama et al. 1978), *S. littoralis* (Kehat et al. 1983, 1986), and *Heliothis virescens* (Tingle and Mitchell 1982). In these cases, even though the mating rate was certainly reduced, field density and crop damage was not significantly decreased. The present study, in which the field population of BAW was remarkably suppressed, is the first case of successful control of noctuid species using sex pheromone.

Effects of Z9E12-14:Ac on *S. litura* population

Spodoptera litura is also a severe pest on the Welsh onion. The major component of BAW sex pheromone, Z9E1214:Ac, is a minor but important component of *S. litura* sex pheromone (Tamaki et al. 1973). Although the mating of *S. litura* has been inhibited by the evaporation of Z9E12-14:Ac (Yushima et al. 1975; Oyama 1977), its effect on the field population was not clear (Oyama et al. 1978).

For the evaluation of the effect of the treatment against *S. litura*, two dry pheromone traps (box type, Takeda Chem. Ind., Ltd. Sato et al. 1978) were set in both treated and untreated areas. An empty trap with no lure was also set 10 m from each trap in the treated area. In order to evaluate the effect on the field population of *S. litura*, the number of egg masses and larvae of *S. litura* were also recorded during BAW field population surveys.

In the treated area, trap catches of *S. litura* males were apparently less than those in the untreated area: 1-15% from late July to early August and 13-30% from mid August to mid September. The field density of the larvae in the treated area seemed to be suppressed in late July and early August (Fig. 3). This suppression was possibly caused by the intensive spray of insecticide against BAW in the treated area. The field density of larvae in the treated area was not remarkably decreased in comparison with that of the untreated area. In the present experiment, the effect of communication disruption with Z9E12-14:Ac, a minor component of *S. litura* sex pheromone, is thought to have been insufficient for reduction of the *S. litura* field population.

Follow-up experiments in 1988

In 1988, we conducted follow-up experiments in which sex pheromone was released at higher rates into smaller areas than in 1987 to reconfirm the population suppression effect. Treated area was about 50 ha of which Welsh onion plots comprised about 24 ha. This area was the northern one-third of the experiment area in 1987. Untreated area was about 9 km from the treated area. Dispensers were set evenly in Welsh onion plots and other cultivated plots at the rate of 1500 and 600 tubes/ha, respectively. Sex pheromone dispensers were set in the plots on 6-8 July and removed on 30 September 1988.

Larval density was surveyed in every six plots in two survey areas in the treated area and in five plots in the untreated area. Surveys were conducted once a week from 13 July through

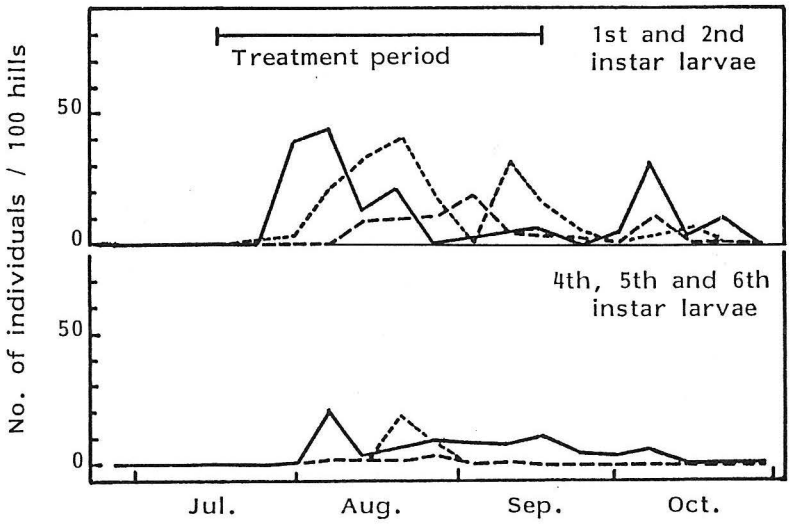

Fig. 3. Densities of *Spodoptera litura* larvae in Welsh onion fields. Broken and dotted lines indicate the densities in the curve areas A and B, respectively. The solid line indicates density in the untreated areas.

27 September. In each plot, the survey was stopped when the number of infested hills reached 25 or when the total number of hills surveyed reached 500.

In the untreated area, densities of egg masses and larvae increased gradually until late August, and rapidly increased in early September (Fig. 4); maximum density of 1st and 2nd instar larvae was more than 400 individuals/100 hills. The mean infestation rate of hills was more than 50% in mid September; in some plots, every hill was observed to be infested by the larvae.

Conversely, population density was low throughout the treatment period in the treated area. No egg mass was found during the treatment period. First instar larvae were found in one survey area on 20 September (0.9 larva/100 hills). No young larvae was found in the second survey area. Few 4th and 5th instar larvae (less than 0.2 individual/100 hills; Fig. 4) were found in both the first (mid-July and late August) and the second survey areas (early September). Infestation rate of hills was less than 0.2% throughout the treatment period.

In 1987, Welsh onion fields were treated with 990 dispensers/ha, and rice fields, greenhouses, orchards, home gardens, and forests were also treated with 320 dispensers/ha. In 1988, 50 ha fields were treated with larger amounts of dispensers than in 1987. The initial density in the treated area was considered to be much lower in 1988 than in 1987, which was indicated by the population survey (Fig. 2, 4) and the capture data with light trap; only a few males were captured throughout the experiment in 1988. Both increased concentration of synthetic sex pheromone and low initial density were thought to have resulted in approximately zero population during the test period. Low initial density was a possible effect of the sex pheromone treatment of the previous year.

It was confirmed again that permeation of the synthetic sex pheromone reduced the egg mass density and consequently reduced the field population of BAW larvae.

Influences Of Delayed Mating

In the open field experiment in 1987, we observed 50-80% of mating ratio in the females caught with the monitoring light trap in spite of drastic reduction in field population density (Table 1). Since the capturing efficiency of virgin females was about one-quarter of that for mated females (Wakamura and Takai 1990), the real mating rate in field population

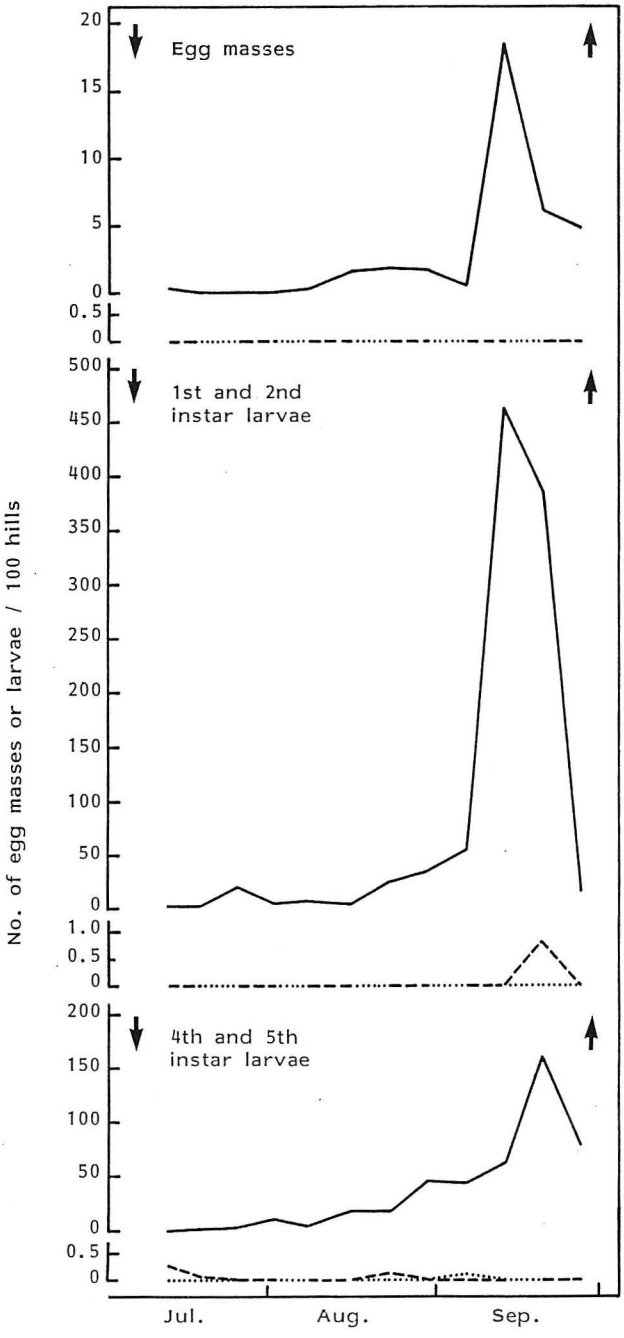

Fig. 4.
Population densities of BAW egg masses and larvae in areas treated and not treated with synthetic sex pheromone in 1988. Broken and dotted lines indicate the densities in two survey areas. Solid line indicates densities in the untreated area. Arrows show the times of setting and removing pheromone dispensers.

was tentatively estimated to be 20-50%. This estimated ratio seems too high to explain the reduction of field population density.

Wakamura et al. (1975) and Barrer (1976) suggested that delay in mating would result in reduction of fecundity in *Cadra cautella*. Kiritani and Kanoh (1984) simulated the expected effect by communication disruption in *Homona magnanima*, based on the reduction caused by delay in mating. In order to understand the effect of mating inhibition, effect of mating delay was investigated in the laboratory (Wakamura 1990).

Delay in mating and reproduction of BAW

One to 10-day-old BAW females were allowed to mate with 2-day-old males. Each pair was placed in a glass pot (9 cm diameter 6 cm high) with a piece of wet cotton. Eggs laid were collected and counted every 3 days and the number of eggs hatched were investigated 4 days after the collection. The insects used were of the 3rd and 4th generations collected from the fields. They were reared on an artificial diet in the laboratory (Wakamura 1988).

The numbers of eggs laid and hatched are shown in Table 2. Three-day-old or younger females laid more than 900 eggs and more than 85% of the eggs hatched. Females older than 4 days laid fewer eggs and their hatchability decreased markedly. The number of unfertilized eggs laid before pairing was increased in 4-day-old and older females. Mating ratios decreased in 8-day-old or older females. The longevity of unmated females was 9.9 days after emergence in a separate investigation.

Delayed mating apparently resulted in decreased reproduction of BAW. Similar phenomena were observed in *Cadra cautella* (Wakamura et al. 1975: Barrer 1976), *Adoxophyes* sp. (Noguchi 1981), *Homona magnanima* (Kiritani and Kanoh 1984) and *Pectinophora gossypiella* (Lingren et al. 1988).

Simulation of reproduction under mating disruption

According to Kiritani and Kanoh (1984), cumulative percentage of mated females and realized expected reproduction (RER) were calculated assuming 90% of mating inhibition. Similar simulation was conducted assuming different percentages of mating inhibition (Table 3). These results suggested that cumulative mating rate and realized expected reproduction would reduce 5 and 30% or less, respectively, when daily mating inhibition were less than 70%. More than 90% reduction of daily mating inhibition is necessary to expect more than 70% reduction of reproduction.

Table 2. Influence of delayed mating on reproduction of BAW female (mean ± SD).

Age (days)	Mating ratio (N)	No. of eggs laid	No. of eggs hatched	Hatchability	Relative fecundity
1	100(10)	991±323	899±372	0.910	92
2	90(10)	1165±248	981±433	0.850	100
3	100(10)	992±271	873±354	0.856	89
4	100(10)	655±217	465±258	0.714	47
6	90(10)	549±204	227±274	0.341	23
8	60(10)	342±252	97±167	0.171	10
10	30(10)	105±148	3±9	0.019	0

Each female of different age was paired with a 2-day-old male.

Table 3. Cumulative ratio of mated females and realized relative expected reproduction in a hypothetical population of BAW.

Daily mating inhibition	Cumulative ratio of mated females	Cumulative realized expected reproduction
0.00	1.00	92
0.20	1.00	93
0.40	1.00	90
0.60	0.99	80
0.70	0.95	71
0.80	0.85	56
0.90	0.59	33
0.95	0.33	18
0.98	0.15	8
0.99	0.08	4

In the 1987 experiment, although a marked reduction of BAW was observed, the mating ratio of females captured with a light trap was 50% or more. Real mating rate was estimated to be 20-50% considering the difference in the trapping efficiency between mated and virgin females (Wakamura and Takai 1990). Applying these estimates to the simulation shown in Table 6, daily inhibition of mating would have been more than 90% throughout the experiment period. In this simulation, removal of females caused by death or dispersal are not considered, which would result in overestimations for both daily inhibition and realized expected reproduction. We can estimate that mating of BAW should have been inhibited 95% or more in the 1987 experiment.

It is therefore concluded that the effect of communication disruption on field populations is the reduction of fecundity caused by mating delay resulting from the reduction of daily mating rate.

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MICROBIAL CONTROL

15

MVP, a Novel Bioinsecticide for the Control of Diamondback Moth

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Abstract

MVP bioinsecticide is the first in a new class of biopesticide products based on Mycogen Corporation's novel CellCap bioencapsulation technology. MVP contains a selected δ-endotoxin of *Bacillus thuringiensis* variety *kurstaki* that is highly active against diamondback moth (DBM), *Plutella xylostella* (L.). Using the CellCap system, this toxin is encapsulated and stabilized within dead bacterial cells that are killed and fixed in the fermentor before harvest using proprietary physical and chemical processes. The effect of the CellCap bioencapsulation process in protecting the δ-endotoxin from environmental degradation was evaluated by applying MVP, and two other *B. thuringiensis* products, Javelin and Dipel 4L to small plots of cabbage and broccoli. Residual activity was estimated using a leaf disc bioassay. These tests demonstrated that MVP provided residual activity that was 2 to 3 times greater than the two other products containing unprotected toxin crystals. Laboratory tests evaluating the effects of ultraviolet radiation on activity of MVP, and other commercial *B. thuringiensis* products such as Dipel, Toarrow-CT and Bacilex demonstrated that persistence of MVP was 5 to 36 times greater than that of other products. Efficacy of MVP was evaluated in small plot tests as well as in large-scale grower trials. In these tests MVP provided consistently superior levels of control of diamondback moth, providing greater efficacy than Javelin, Dipel 4L and Cutlass and levels of control equivalent or superior to those achieved with standard chemicals.

Introduction

Insecticide resistance has become a worldwide problem in the control of diamondback moth (DBM), *Plutella xylostella* (L) (Lepidoptera: Yponomeutidae) (Sun et al. 1978; Liu et al. 1981, 1982; Miyata et al. 1982; Chen and Sun 1982; Tabashnik et al. 1987; Magaro and Edelson 1990), and represents a serious threat to the effective management of this important crucifer defoliator. The high degree of efficacy, rapid kill and ease of use of broad-spectrum chemical compounds like the pyrethroids have been a strong incentive to growers to use them exclusively and as preventive measures to control pest populations. The resulting reliance on this single approach to pest control has led to ever-increasing application rates, decreasing effectiveness and eventual breakdown of control.

In general terms there are other problems associated with the dominant use of broad-spectrum chemical insecticides. In addition to insecticide resistance, the effect of these compounds on beneficial insect populations frequently results in secondary pest outbreaks and pest resurgence, and is increasingly recognized as adversely affecting our ability to manage pest populations in general. In many different crop situations in the United States, pesticide residues, groundwater

*MVP and CellCap are trademarks of Mycogen Corporation, San Diego, California, U.S.A.

contamination, worker safety, and waste disposal are other pesticide-related issues of growing concern to the agricultural community and the general public alike.

The need for alternative strategies to mitigate these side effects of dependence on broad-spectrum insecticides has been noted many times, and led to the concept of integrated pest management (IPM) (Smith and Reynolds 1966; Smith and van den Bosch 1967). The development of economic injury levels and action thresholds and at least the first stages of progress toward implementing IPM programs has now occurred in many different crop situations, including crucifers.

However, the development of efficacious, selective alternative control methods and products to use in IPM programs is essential for these programs to be successful. Among the most promising of these alternatives are products based on insect pathogens and naturally occurring insect toxins. These products, commonly called bioinsecticides or microbial insecticides, are effective and specific for target pests, offering a great deal of promise in resolving some of the problems commonly associated with dependence on broad-spectrum insecticides. In addition, by offering a completely different mode of action from conventional neurotoxic chemical insecticides they can be very useful in managing resistance to these chemicals.

Bacillus thuringiensis bioinsecticides

The most successful of the bioinsecticide products developed to date are those based on *Bacillus thuringiensis* Berliner (*B.t.*), a spore-forming bacteria that produces a selectively toxic protein in the form of an inclusion or crystal within the cell. This protein inclusion is the active component in *B.t.* products and consists of the protoxin form of one or more α-endotoxins. When this inclusion is ingested by a susceptible insect host, it is solubilized and the protoxin processed to the active δ-endotoxin form. This active toxin then binds to and destroys the midgut epithelium, causing a rapid gut paralysis and cessation of feeding in less than an hour after ingestion in the most sensitive species. Death generally occurs within 1-3 days.

Limitations of *B.t.* bioinsecticides

Effective commercial *B.t.* products for the control of caterpillars have been available for over 20 years and have been used against caterpillar pests of cruciferous crops for many years. However, conventional *B.t.* products have some serious limitations. One of the most significant of these is the short residual activity under field conditions. This lack of foliar persistence is a key factor in the inconsistent performance of conventional *B.t.* insecticides. This factor coupled with low efficacy on certain key target pest species is largely responsible for *B.t.* products not being more widely used.

These products are all manufactured using standard fermentation techniques. During the fermentation of *B.t.* cells, spores and toxin crystals are produced and released into the medium when cell walls lyse at the conclusion of the fermentation cycle. It is these spores and crystals which constitute the active ingredient in conventional *B.t.* products. When growers apply a conventional *B.t.* insecticide to their crop, they are applying these spores and naked crystals of *B.t.* It is these unprotected toxin crystals that are so susceptible to degradation (Fig. 1A). Ultraviolet radiation (UV) has been shown to rapidly degrade the activity of *B.t.* products (Ignoffo et al. 1977; Morris 1983; Sneh and Schuster 1981). In a given crop, the bulk of activity can be lost within 2-3 days. The insecticidal half-life of *B.t.* products exposed to direct sunlight has been estimated to be 1.5-2 days on cotton foliage (Beegle et al. 1981) and 2 days on white spruce (Morris and Moore 1975). This means a lethal dose is present for only a relatively short time, decreasing efficacy and making frequent applications necessary.

CellCap delivery system

The CellCap bioencapsulation and delivery system is Mycogen's proprietary technology for enhancing the field persistence and efficacy of *B.t.* toxins. The development of a new

biopesticide product based on this CellCap process actually begins when a *B.t.* isolate with the desired host range and level of potency is identified in a focused screening and bioassay effort against a particular target pest. The gene(s) coding for the desired δ-endotoxin(s) is then isolated and transferred into a *Pseudomonas fluorescens* host isolated from the phylloplane. In the manufacture of a CellCap product the transformed *P. fluorescens* cells are cultured in large-scale fermentors. Unlike *B.t.* cells which lyse at the end of the fermentation cycle, the *P. fluorescens* cell walls remain intact. The *P. fluorescens* cells are then killed in the fermentor before harvest using a proprietary physical and chemical process. This process also fixes the cell wall by cross-linking cell wall components, creating a stable, dead cell biocapsule which encapsulates and protects the toxin crystal. Thus, the active component of any CellCap product contains no living cells, but rather consists of a selected toxin (or toxins) encapsulated within a dead cell biocapsule (Fig. 1B).

MVP is the first of the CellCap products, and has been developed for the control of lepidopterous pest larvae. A δ-endotoxin from *B.t.* variety *kurstaki* with high activity against DBM and a number of other key caterpillar pests in vegetable crops, was selected for this product. The studies reported here were conducted to evaluate MVP and assess the effect of the CellCap bioencapsulation technology on the efficacy and foliar persistence of *B.t.* toxins.

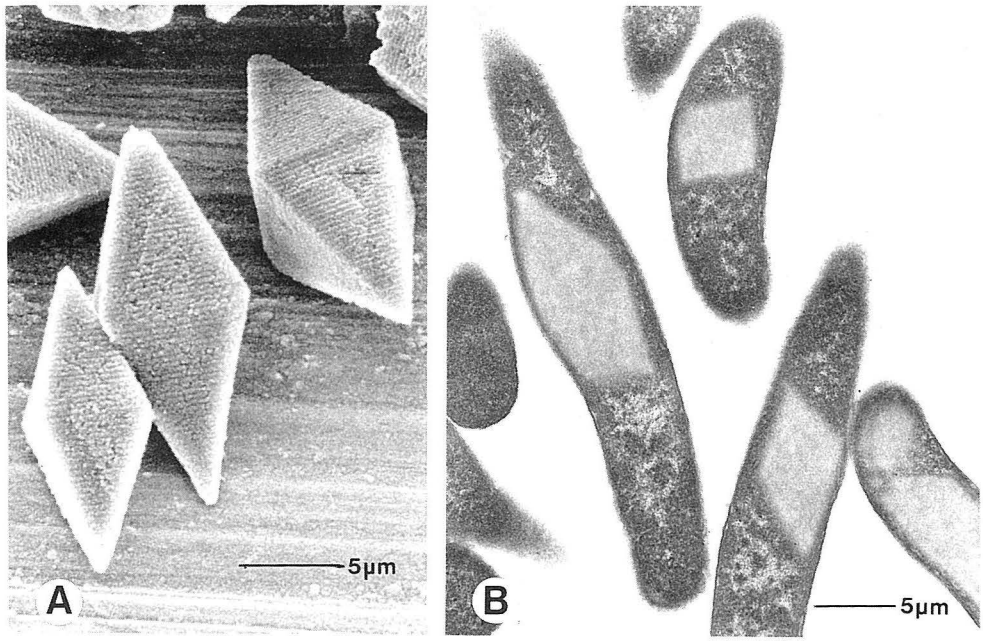

Fig. 1. Comparison of different delivery systems for *B.t.* δ-endotoxins as they would appear after being applied to foliage: A) a toxin crystal produced in the natural *B.t.* in a conventional *B.t.* product; B) toxin crystals produced in the CellCap product, MVP, encapsulated within dead cells of *Pseudomonas fluorescens*.

Materials and Methods

MVP persistence studies

Foliar persistence tests. Foliar persistence of MVP was evaluated in small plot tests in 1988 and 1989 in the states of Florida, California, Wisconsin and North Carolina. A total of

20 studies were conducted. The experiments were conducted in small plots of broccoli or cabbage treated with MVP and two representative registered *B.t.* products, Javelin (Sandoz Crop Protection Corp.) and Dipel 4L (Abbott Laboratories). Applications were made at label rates of each product or 20 BIU (billion international potency units)/ha. Toxin equivalence was estimated for each product using a gel scan quantification technique. Test materials were applied either with a CO_2 backpack sprayer or tractor-mounted boom sprayer, using one overhead and two drop hollow cone nozzles (Spraying Systems TXUS8). Leaf disc samples (200 discs per treatment) were collected at several post-treatment intervals beginning with the day of treatment and ending at 12 days. These leaf discs were brought back to the lab and bioassayed placing a single third instar DBM larvae on each disc and evaluating mortality 4 days after infestation. The method developed for these tests has proven to be a highly sensitive technique for quantifying residual *B.t.* activity.

UV degradation experiments. The effect of UV on the activity of MVP, and the conventional *B.t.* products Dipel, Toarrow-CT (Toagosei Chemical Co.) and Bacilex (Shionogi Pharmaceutical Co.) was also examined. In these tests, 10 ml aqueous supensions of each of the test products were placed in open petri dishes on a flask shaker. These dishes were then gently agitated while being exposed to 315 nm UV lamps and 350 nm UV black-light lamps simultaneously inside a wooden box fitted over the shaker. UV exposure was estimated at 0.6 mw/cm^2, or a rate approximately six times that of sunlight. Suspensions were agitated slowly during exposure and samples were collected at 0, 1, 2, 3, and 6 hours. Control samples were covered with foil. All samples were bioassayed against third instar DBM using a diet incorporation method. Five separate experiments were conducted and LC_{50} values calculated for each of the samples.

MVP Field Efficacy Evaluations

Small plot tests. MVP was widely tested in 1988, 1989 and 1990 against DBM in the United States. Small plot tests on cabbage, broccoli and other crucifers were conducted in areas where DBM resistance to pyrethroids is widespread as well as areas where resistance is less of a problem. Test locations included sites in the Rio Grande Valley of Texas, Florida, California, North Carolina and parts of the midwestern and northeastern United States. All tests included competitive *B.t.* products, such as Javelin and Dipel 4L, as well as at least one standard chemical treatment generally utilized by growers in the particular region. Applications were made every 4-7 days throughout the crop cycle. Label rates of registered products and equivalent BIU rates of MVP were applied and toxin equivalence estimated as in the persistence studies. Plants from each plot were destructively sampled every 5-7 days and complete larval counts made. Yield evaluations were also made at harvest.

In 1989, 18 small plot tests were conducted on cruciferous crops in 11 states. In 1990, 40 small plot tests were conducted in 16 states.

On farm tests (EUP trials). In 1990 the United States Environmental Protection Agency (EPA) granted Mycogen an Experimental Use Permit (EUP) for MVP, allowing large-scale on-farm tests to take place in key locations throughout the United States. These grower trials were conducted on cruciferous crops on 80 farms in 10 states on a total of 880 ha. Growers used MVP and their standard insecticide treatments in side-by-side comparisons. Cooperating growers were surveyed after the trials and were asked to evaluate the performance of MVP compared with their standard insecticides.

Results

MVP Persistence Studies

Foliar persistence tests. The CellCap system was found to consistently enhance foliar persistence of *B.t.* δ-endotoxin when compared to other *B.t.* products. Generally, within the

first 2 days after application, insecticidal activity of Dipel and Javelin had dropped below 80% of the original activity. In contrast, when an equivalent rate of δ-endotoxin was delivered in MVP, insecticidal activity remained close to 100% of the original level after 4 days. By 7 days residual activity of MVP was still above 70%, while both Javelin and Dipel had dropped below 50%. In 20 studies conducted in four different geographic regions, MVP consistently provided high levels of residual activity 2-3 times greater than those recorded for Javelin and Dipel 7 days after treatment. The results of 2 years of foliar persistence studies are summarized in Table 1. The results of two specific trials, one conducted in southern California under hot, dry conditions and the second conducted in the hot, humid climate of central Florida are given in Fig. 2. In both locations MVP showed substantially enhanced persistence that was respectively 2 and 3 times greater than that of Dipel and Javelin in California and 2 and 9 times greater in Florida.

Table 1. Summary of foliar persistence studies of MVP and other *B.t.* products, Javelin and Dipel 4L; residual activity determined 7 days after treatment using bioassay on DBM third instar larvae.

Product	Rate[a] (g toxin/ha)	% DBM Mortality[b]		Persistence[c] Index	
		1988	1989	1988	1989
MVP	45	75	76	100	100
Dipel	45	48	40	64	52
Javelin	74	37	27	49	35

[a]based on gel scan of product. [b]Average % mortality at 10 study locations per year.

[c]Persistence index = $\frac{\%\text{ DBM mortality in treatment}}{\%\text{ DBM mortality in MVP treatment}} \times 100$

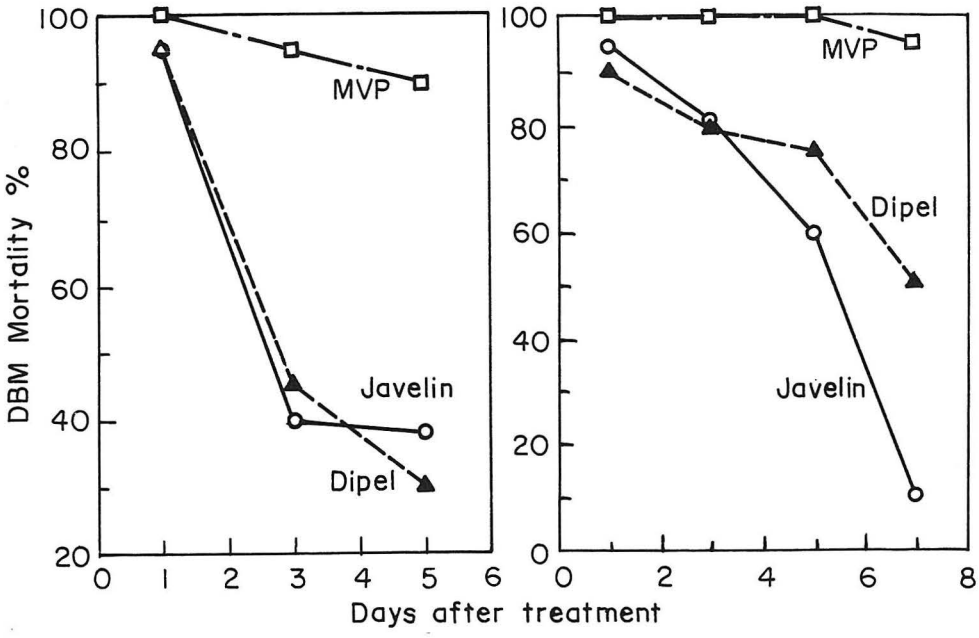

Fig. 2. Comparison of foliar residual activity of MVP and two commercial *B.t.* products, Dipel 4L and Javelin, at two climatically different regional locations; applications made at equivalent 45 g δ-endotoxin/ha. Left, Irvine, California, on broccoli. Right, Sanford, Florida, on cabbage. Residual activity assessed using bioassay of leaf discs removed and fed to third instar DBM at several post-treatment intervals, with mortality determined 4 days after infestation.

UV Degradation Experiments. Activity decreased in all treatments receiving the combined 315 nm and 350 nm UV over the 6 hour time course of these experiments (Fig. 3). However, MVP had virtually no loss in activity after 3 hours, and after 6 hours had still retained 61.4 ± 12.2% of its original activity. Dipel and Toarrow-CT decreased steadily in activity over the first hours and had only 12.2 ± 4.0% and 12.8 ± 10.2% of the original activity remaining at 6 hours. Bacilex showed the greatest sensitivity to degradation under these conditions with only 1.7 ± 1.3% retained after the same period of exposure.

The results of these experiments showed that a combination of 315 nm and 350 nm UV can cause rapid degradation of *B.t.* activity and that the CellCap delivery system used in MVP protects the δ-endotoxin from degradation by these wavelengths of UV radiation, providing far greater persistence of the activity when compared with other commercial *B.t.* products. These results corroborated the field foliar persistence results.

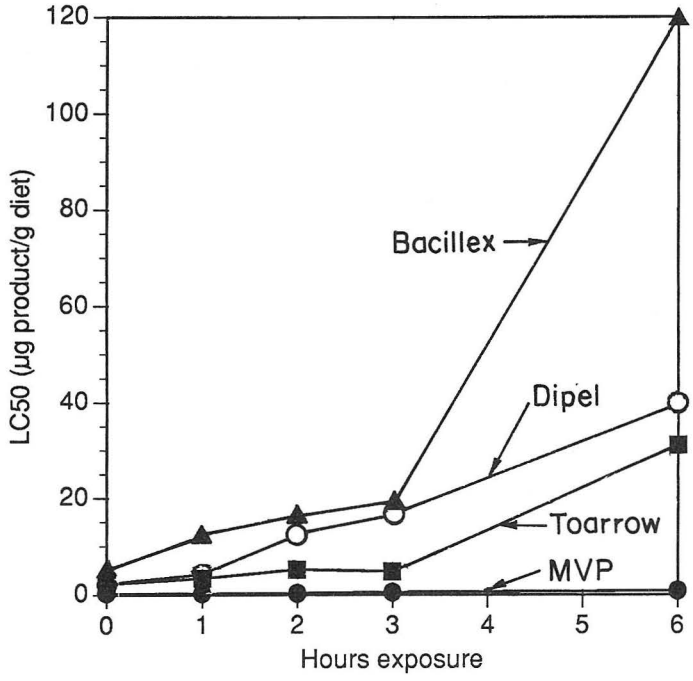

Fig. 3. Persistence of MVP and three other conventional *B.t.* products determined in laboratory experiments. Product suspensions were exposed to 315 nm and 350 nm UV radiation for 6 hours and bioassayed against third instar DBM at several intervals after treatment. Potency loss expressd as increase in the LC50.

MVP Field Efficacy

Small plot trials. MVP provided excellent control of DBM in small plot tests conducted between 1988 and 1990. Typically MVP outperformed all other *B.t.* products and provided consistently superior levels of control of DBM (Table 2). These results demonstrated that for control of DBM, the unique CellCap bioencapsulation and delivery system provided greater efficacy than any other biopesticide product tested, as well as levels of control equivalent to or superior to those achieved with standard chemical treatments (Tables 2).

On-farm tests (EUP trials). Control of DBM in these trials was excellent, with MVP consistently outperforming a range of other insecticide products. The most vivid and impressive measure of these results was the response of cooperating growers. Of the growers surveyed, 85% said that MVP provided excellent control of DBM that surpassed the performance of their standard insecticides. A full registration and market introduction of MVP in the United States is expected in 1991.

Table 2. Efficacy of MVP and two other *B.t.* products for control of DBM larvae on cabbage. Texas, USA 1990.

Treatment	Rate product/ha	Average[a] DBM larvae/5 plants[b]
MVP	5.0 l	15.0 a
Javelin	1.5 kg	115.5 c
Cutlass	2.3 kg	50.0 b
Untreated	–	190.3 d

[a]Means flanked by the same letter are not significantly different (DMRT, $P = 0.05$)
[b]Average number of larvae determined from total counts on 5 plants / replicate (20 total/treatment) destructively sampled 7 days after a single treatment.

Discussion

The results obtained in the studies reported here have demonstrated that the CellCap bioencapsulation and delivery system can be used to produce very efficacious, longer residual bioinsecticides. The increased residual activity of MVP, coupled with its selected toxin with high activity against DBM, has proven to be a highly effective combination. MVP performance against DBM has consistently surpassed conventional *B.t.* products, and has proven to be as good or better than the standard synthetic chemical treatments used in cruciferous crops. This increased efficacy is due to the use of selected toxin(s) and increased foliar persistence over conventional *B.t.* products, made possible by the bioencapsulation delivery system used in MVP which protects and stabilizes *B.t.* toxins. In addition because MVP is based on a dead cell encapsulated toxin and not on living cells, many self-life stabilization problems are avoided. In the production of MVP, the CellCap process creates a highly stable active ingredient.

The concept of IPM stresses the need to understand and build on existing ecological processes and key interspecific relationships that are present in particular agroecosystems and utilizing these to maximum advantage in regulating and managing pest populations. This construction of pest management systems from the bottom up, building on this foundation of existing ecological factors, is still a long way from being the primary approach in pest management, and in most crop systems we are at best still in the very earliest stages of IPM development. It is clear that as these more sophisticated and presumably more successful IPM systems are developed, the availability and use of selective control tactics will become increasingly important as management tools that are effective and yet also compatible with existing natural enemies and other ecological factors that contribute to regulation of pest populations. *B.t.* δ-endotoxin-based products like MVP are among the most effective of the selective insecticides that are currently available, and among the least disruptive to the existing natural enemy complex, therefore minimizing the potential for pest resurgence and secondary pest outbreaks. In this regard they can fill a key niche in many IPM programs by offering effective yet selective action, a benefit which is not offered by many other products.

In cruciferous crops the successful use of *B.t.* in the control of lepidopterous larvae without disruption of the natural enemy complex was first demonstrated over 10 years ago (Kennedy and Oatman 1976; Wyman and Oatman 1977). In a 3-year study in cabbage in Wisconsin, Quick and Wyman (in preparation) examined the effect of broad-spectrum compounds (fenvalerate and metamidophos) and *B.t.* (Dipel 4L) on control of DBM, imported cabbageworm, *Pieris rapae* L., and cabbage looper, *Trichoplusia ni* (Hübner) and the effect on parasitism. They found that the *B.t.* treatment regime provided acceptable levels of control while having little or no effect on levels of parasitism, which did not generally differ significantly from levels observed in untreated plots. The broad-spectrum insecticide treatments showed direct toxicity to parasites and levels of parasitism that were significantly lower in general. Sudarwohadi et al. (1977) and Sastrodihardjo (1986) noted that *B.t.* applications in Indonesia effectively controlled DBM without harm to the ichneumonid *Diadegma eucerophaga* Horstm. Lim et al. (1986) used chemical

exclusion techniques in small plot tests against DBM on cabbage to show the relative impact of different pesticide compounds on *Apanteles plutellae*. In these tests sevithion (carbaryl + malathion), which was toxic to the parasitoid but not DBM, reduced *A. plutellae* populations to levels well below those of control and Dipel-treated plots. At the same time DBM populations resurged to levels over five times those of the control plots. In the Dipel treatment much higher levels of *A. plutellae* were recorded than in any other treatment.

MVP is unique in providing substantial improvements in efficacy against DBM, with performance equivalent or superior to that offered by broad-spectrum insecticides, while providing the benefits of selective activity that can minimize disruptions and preserve natural control systems essential to the long term management of DBM populations.

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16

Microbial and Other Insecticides to Control Lepidopterous Pests of Cole Crops in Georgia

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Abstract

Synthetic and microbial insecticides were evaluated from 1988 to 1990 to control an insecticide-resistant population of diamondback moth, *Plutella xylostella* (L.), and the cabbage looper, *Trichoplusia ni* (Hübner), on cabbage and collard. The pyrethroid insecticides tested including permethrin and esfenvalerate were generally incapable of controlling the diamondback moth although high rates produced more than 95% marketable heads in some tests. Permethrin was synergized with piperonyl butoxide. The organophosphorus insecticide mevinphos, which has been used in the area for more than 25 years, still contolled *P. xylostella*. Microbial insecticide (*Bacillus thuringiensis* Berliner) products Dipel, Javelin, Cutlass, and MVP were moderately effective against both pests but needed short (4-5 days) application intervals during severe pest pressure and environmental conditions as occur during the summer season in Georgia. Two molting inhibitors, teflubenzuron and flucycloxuron, were effective against the diamondback moth but less so against the cabbage looper.

Introduction

Cole crops in Georgia are infested with a complex of lepidopterous defoliators. Most serious are the cabbage looper (CL), *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) and the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae). Until 1987 the insecticides of choice for controlling these pests in Georgia were the pyrethroids, esfenvalerate and permethrin. Resistance in the DBM to these materials in Georgia was first recognized in 1987 and an 80-fold resistance to permethrin was later corroborated by Wyman (personal communication *in* Adams et al. 1990). The following report is a result of 3 years evaluation of potential replacement insecticides for management of these pests in cabbage (*Brassica oleracea* var. *capitata*) and collards (*B. oleracea* var. *acephela*). Except for the formation of a head by cabbage, both crops are similar and have identical pests.

Methods and Results

General methods

Insecticides were applied with a two-row tractor-mounted boom sprayer equipped with three Spraying Systems No.T-18 hollow cone nozzles per row directed above and along both sides of the plants. Delivery was 467 l/ha at a pressure of 3.52 kg/cm^2 (50 psi). Plot size was two rows, 0.3 m wide, and 15-23 m long. Treatments were replicated four times in randomized complete blocks. Three to four days after insecticidal application, living larvae were recorded on five plants per plot. When plants were ready to harvest, 10 plants per plot were scored for damage using a 0-5 scale in which 0 to 2 = no, light or moderate damage to the outer leaves;

3 to 5 = light, moderate, and severe damage, respectively, to the head of cabbage or central portion of collards. Plants were divided into two market-quality categories based on variable market standards. Best were those with scores of 0 to 2. Fair were those with scores of 0-3. Plants with scores of 3 would not be marketable under surplus conditions. Cultivars were 'Rio Verde' cabbage and 'Vates' collards.

Data were subjected to ANOVA (SAS 1985) and significant difference ($P = 0.05$) between means is the LSD as determined by Waller-Duncan K-Ratio T-Test (Waller and Duncan 1969).

Test 1, 1988

Cabbage transplanted 6 April 1988 was treated on 10, 17, and 25 May and 9 June. On 15 June, plants were scored for damage and living larvae were recorded (Table 1). Only DBM were in significant numbers. On cabbage only treatments with Dipel ES (*Bacillus thuringiensis*, var. *kurstaki* 17,600 IU/mg, Abbott Laboratories) had larval counts significantly lower than the untreated check. Damage scores and market quality (% fair heads) for these treatments and permethrin (0.11 kg AI/ha) were also significantly better than the check. Fenoxycarb, a molting inhibitor, significantly increased the effectiveness of Dipel. Larval counts for the other insecticides, including the pyrethroids bifenthrin, esfenvalerate and cyhalothrin were not significantly different from the check. Counts for cyhalothrin (0.011 kg AI/ha) and thiodicarb (0.84 kg AI/ha), a carbamate, were significantly higher than the check. Damage scores for bifenthrin, cyhalothrin, esfenvalerate and thiodicarb were equal or poorer than the check.

Table 1. Feeding damage and DBM larval infestation of cabbage treated[a] with insecticides. Test 1. Tifton, Georgia, 1988.

Treatment	L or kg/ha	Damage score[b]	No. DBM/ 5 plants	%Marketable[c]	
				Best	Fair
Dipel ES	1.2	2.7	0.3	40.0	95.0
Dipel ES	0.6	3.0	2.0	35.0	65.0
Dipel ES + fenoxycarb 25WP	1.2+.14	2.2	1.3	60.0	100.0
Dipel ES + fenoxycarb 25WP	0.6+.14	1.7	1.0	95.0	100.0
Bifenthrin 10 WP	0.11	3.1	6.8	20.0	75.0
Bifenthrin 10 WP	0.056	4.4	14.0	0.0	0.0
Cyhalothrin 1EC	0.022	3.9	26.3	0.0	20.0
Cyhalothrin 1EC	0.011	3.9	33.5	0.0	20.0
Esfenvalerate 0.66 EC	0.07	3.4	22.8	0.0	65.0
Esfenvalerate 0.66 EC	0.03	3.5	19.3	5.0	50.0
Permethrin 3.2 EC	0.11	2.5	8.5	55.0	100.0
Thiodicarb 3.2 EC	1.68	3.6	15.5	0.0	40.0
Thiodicarb 3.2 EC	0.84	3.9	30.3	0.0	10.0
Dipel ES + esfenvalerate	1.2+.07	2.6	4.0	20.0	100.0
Dipel ES + esfenvalerate	1.2+.03	2.3	4.3	70.0	100.0
Dipel ES + mevinphos 4 EC	1.2+1.1	1.9	0.3	75.0	100.0
Dipel ES + methomyl 2.4LV	1.2+1	2.1	4.0	70.0	100.0
Dipel ES + naled 8 EC	1.2+2.2	2.6	2.8	35.0	100.0
Methamidophos 4 EC prehead, Dipel ES+mevinphos post head	1.1 followed by 1.2+0.6	2.8	0.3	20.0	100.0
Untreated check		3.6	16.0	0.0	45.0
LSD (p=0.05)		0.7	11.8	36.9	31.5

[a]Treated 10, 17, 25 May and 9 June, evaluated 15 June. [b]Score, 0-5 damage scale: 0 = no, 1-2 = light to moderate to outer leaves, 3-5 = light to severe to head and wrapper leaves. [c]Marketable: Best = heads with scores 0 to 2, fair = heads with scores 3 or less.

Test 2, 1989

Cabbage was transplanted on 5 April. An adjuvant, Bond (Loveland Industries, Inc), was used with all treatments at a concentration of 0.25%. Treatments were applied 4, 12, 18, and 26 May and 1 June. Insects were recorded on five plants/plot on 8, 15, and 22 May, and 7 June. The CL was the most prevalent caterpillar and reached a population peak on 15 May. DBM occurred in much smaller numbers. Larval counts (average of four dates) for the CL and DBM, damage scores and market quality are shown in Table 2. Fewest larvae were recorded in plots treated with esfenvalerate (0.06 kg AI/ha), Cutlass OF (*Bacillus thuringiensis* Ecogen, Inc., Langhorne, PA) (4.67 l/ha), all rates or Cutlass WP, and all rates of AC30360 (American Cyanamid Co., Princeton, N.J.). Feeding damage scores were lowest and percent marketable heads were highest in plots treated with AC30360 (0.36 kg AI/ha). Other treatments producing more than the 80% marketable heads (fair grade) included Cutlass OF (4.67 l/ha), Cutlass WP (1.12 and 2.24 kg/ha), Dipel WP (1.12 kg/ha) and flucycloxuron (Uniroyal Corp) (0.14 kg AI/ha). Note that in this test the WP formulations of Cutlass and Dipel gave more marketable heads than the comparable liquid formulations. The experimental field received 12 irrigations and rainfalls for a total of 340 mm.

Table 2. DBM, CL and market quality of cabbage treated[a] with insecticides, Tifton, Georgia, Test 2, 1989.

Treatment	Liters/ kg AI/ha	No./5 Plants[b]		Damage score[c]	% Marketable[d]	
		DBM	CL		Best	Fair
Cutlass OF	4.67	0.3	2.1	2.4	60.0	92.5
Cutlass OF	2.34	0.5	3.7	3.3	5.0	62.5
Cutlass OF	1.17	1.0	4.3	4.0	0.0	12.5
Cutlass WP	2.24	0.3	2.1	2.4	60.0	92.5
Cutlass WP	1.12	0.6	2.1	2.6	52.0	85.0
Cutlass WP	0.56	0.6	1.8	3.1	12.5	72.5
Dipel 2X WP	0.56	0.8	2.3	2.6	37.5	92.5
Dipel ES	1.17	0.4	2.5	3.1	15.0	72.5
Esfenvalerate 0.66E	0.06	0.3	1.5	2.6	50.0	90.0
AC303630 1EC	0.12	0.1	1.4	3.1	30.0	65.0
AC303630 1EC	0.24	0.1	0.6	2.1	75.0	95.0
AC303630 1EC	0.36	0.4	0.6	1.6	90.0	100.0
Flucycloxuron 2L	0.14	0.3	2.3	2.7	37.5	87.5
Diflubenzuron 25W	0.14	0.9	5.9	4.9	0.0	0.0
Untreated Check		3.1	7.6	5.0	0.0	0.0
LSD (P = 0.05)		0.2	1.7	0.6	33.5	21.3

[a]Treated 4, 12, 18, 26 May and 1 June. [b]Average of 8, 15, 22 May and 2 June counts. [c]Score, 0-5 damage scale: 0 = no, 1-2 = light to moderate to outer leaves, 3-5 = light to severe to head and wrapper leaves. [d]Best = heads with scores 0 to 2, Fair = heads with scores 3 or less.

Test 3, 1989

Collard was transplanted on 5 May. Bond was used with all materials as above. The treatment interval beginning 26 May was about 7 days except for MVP (encapsulated delta endotoxin of *B. thuringiensis* var. *kurstaki*, Mycogen, Corp. San Diego, CA.) and Javelin (*B.thuringiensis* var. *kurstaki*, Sandoz Corp. Des Plains, IL) which were compared in 4-, 7- and 10-day schedules. Insects were recorded on five plants/plot on 31 May, 8, 15, 22 and 29 June, and 27 July.

The principal defoliating caterpillar was the CL. Populations of both CL and DBM were highest on 8 and 29 June. Larval numbers averaged across six dates are given in Table 3. Fewest total larvae (1.75) were recorded in plots treated with Cutlass OF (4.67 l/ha) applied at 7-day intervals. This treatment was not significantly different from MVP (2.34 or 4.67 l/ha), applied

at 4-day intervals, or Javelin (2.34 l/ha) applied at 4-, 7- or 10-day intervals, Cutlass WP (2.24 kg/ha), Dipel ES (2.24 l/ha), Dipel 2X (1.12 kg/ha), and permethrin (0.11 kg/ha) all applied at 7-day intervals.

Average damage scores and percent marketability (plants with scores <3) are shown in Table 3. Lowest scores and most marketable plants are in plots treated with permethrin (0.22 kg AI/ha) or flucycloxuron followed by permethrin (0.22 kg AI/ha) or esfenvalerate (0.034 kg AI/ha), and Javelin (4.67 l/ha, 4-day interval). Addition of Ridomil/Bravo (81% metalaxyl + chlorothalonil, CIBA-GEIGY Corp) to or removal of Bond sticker from MVP treatment had no significant effect on damage and marketability.

Table 3. DBM, CL, damage and market quality of collard treated with insecticides. Tifton, GA. Test 3 1989

Treatment	L or kg/ha/ Schedule[a]	No./5 Plants[b]		Score[d] 7/9	% Marketable[e]
		DBM	CL		
MVP	4.67/4	0.6	4.8	3.6	37.5
MVP	4.67/7	0.4	5.8	4.0	2.5
MVP	4.67/10	0.7	5.4	4.6	5.0
MVP	2.34/4	0.3	3.6	3.9	15.0
MVP	2.34/7	1.3	9.0	4.0	7.5
MVP	2.34/10	0.8	5.3	4.8	7.5
MVP + Ridomil/Bravo	4.67 + 2/7	1.1	6.3	4.0	20.0
MVP, without Bond	4.67/7	0.7	5.6	3.7	25.0
Javelin	4.67/4	0.5	3.5	3.3	67.5
Javelin	4.67/7	0.8	3.7	4.0	12.5
Javelin	4.67/10	0.7	4.5	4.2	2.5
Esfenvalerate .66 EC	0.03/7	1.8	4.5	3.2	55.0
Cutlass OF	4.67/7	0.4	1.3	3.5	45.0
Cutlass WP	2.24/7	0.7	2.8	3.8	17.0
Dipel ES	2.34/7	0.4	2.5	3.7	25.0
Dipel 2X WP	1.12/7	1.2	3.9	3.9	20.0
Permethrin 3.2 EC	0.11/7	2.0	3.4	3.4	60.0
Permethrin 3.2 EC	0.226	–	–	2.9	90.0
Flucycloxuron 2L/[c]	0.14				
Esfenvalerate	0.03/7	1.5	6.3	2.7	100.0
Flucycloxuron 2L/[c]	0.14				
Permethrin	0.22/7	2.0	4.6	2.5	97.5
Diflubenzuron 25 WP	0.28/7	2.9	4.3	4.2	25.0
Untreated Check		3.6	12.0	4.2	17.5
LSD (P = 0.05)		1.4	3.2	0.6	36.7

[a]4 day: 26, 29 May , 2, 5, 12, 16, 19, 23, 26, 30 June, 3 July. 7 day: 26 May, 2, 12, 19, 27 June , 3 July. 10 day: 26 May , 5, 16, 26 June , 3 July. [b]Average of 31 May, 8, 15, 22, 29 June , 7 July counts. [c]Flucycloxuron applied 1st two applications only. [d]Score, 0-5 damage scale: 0 = no, 1-2 = light to moderate to outer leaves, 3-5 = light to severe to head and wrapper leaves. [e]Plants with damage scores of 0 to 3.6 Treated 12, 19, 27 June, 3 July.

Test 4, 1989

Cabbage was transplanted on 6 September 1989. Insecticides were applied on 20 and 27 September, 4 and 16 October, 2 and 10 November. Insects were recorded on five plants/plot on 2 and 10 October, and 11 November. Plants were scored for damage on 13 November. The CL was predominant on 3 October (data not shown). DBM and CL reached moderate numbers on 13 November (Table 4). All treatments except esfenvalerate, 0.03 kg AI/ha, significantly reduced larval numbers below the check. Damage scores made 17 November are shown in Table 4. Highest percentages of best plants (scores of 2 or less) were obtained with permethrin (0.11 kg AI/ha) synergized by piperonyl butoxide (PBO) at 0.6 kg AI/ha; MVP 4.67 l/ha,

Table 4. DBM, CL, damage and market quality on cabbage treated[a] with insecticides, Tifton, Georgia. Test 4, 1989.

Treatment	L or kg/ha	No./5 Plants[b]		Damage score[c]	% Marketable[d]	
		DBM	CL		Best	Fair
Permethrin 2 EC	0.11	1.8	0.0	3.3	10.0	65.0
Permethrin 2 EC + PBO 8E	0.1 + 0.6	0.0	0.0	2.0	78.0	98.0
MVP	4.67	0.5	0.0	2.1	78.0	100.0
Cutlass OF	4.67	0.3	0.0	2.1	60.0	100.0
Javelin WG	1.12	0.5	0.3	2.7	35.0	98.0
Dipel 2X WP	1.12	0.8	0.3	3.2	5.0	48.0
Esfenvalerate 0.66 EC	0.03	4.5	0.0	2.8	28.0	85.0
Chlorpyrifos 50 WP	0.56	0.5	1.3	4.0	0.0	8.0
Chlorpyrifos 50 WP	1.12	3.5	6.8	5.0	0.0	2.0
Chlorpyrifos + Javelin WG	0.6 + 0.6	0.5	0.5	3.6	5.0	38.0
Chlorpyrifos + Javelin WG	0.6 + 1.1	0.5	1.8	3.0	25.0	80.0
Chlorpyrifos + Javelin WG	1.1 + 1.1	0.8	1.0	2.6	40.0	90.0
Chlorpyrifos + Dipel 2X	1.1 + .6	1.0	1.0	3.4	10.0	45.0
Chlorpyrifos + Javelin + Mancozeb 80 WP	1.1 + 1.1 1.8	0.8	0.8	2.4	52.0	85.0
Chlorpyrifos + Javelin WG + Ridomil/Bravo 81 WP	1.1 + 1.1 1.8	0.5	2.5	3.3	2.5	68.0
Untreated Check		12.5	14.5	5.0	0.0	0.0
LSD (P = 0.05)		5.2	5.3	0.5	26.5	21.3

[a]Treated 20, 27 September, 4, 16 October, 10 November. [b]11 November. [c]Score, 0-5 damage scale: 0 = no, 1-2 = light to moderate to outer leaves, 3-5 = light to severe to head and wrapper leaves. [d]Best = heads with scores 0 to 2, Fair = heads with scores 3 or less.

and Cutlass 4.67 l/ha. Eight treatments produced more than 79% good plants (scores 3 or less). Addition of mancozeb or Ridomil-Bravo to a chlorpyrifos Javelin mixture did not cause phytotoxicity; however, Ridomil-Bravo reduced the efficacy of the mixture from 40 to 2.5% best plants and from 90 to 68% good plants.

Test 5, 1990

Cabbage was transplanted 27 March 1990. Insecticides were applied 23 April, 1, 10, 16 and 23 May 23. Bond was used with all materials (except the oil combination) at a concentration of 0.25% for the first two applications. Because Bond tended to clog the nozzles, 0.25% Valent X-77 (Valent Corp.) spreader was used for the final applications. Rainfall and irrigation during the test period totaled 116 mm.

The DBM was the only significant insect pest on the cabbage. Averages for counts made 30 April, 7, 14 and 27 May are shown in Table 5. Fewest larvae, lowest damage scores and most marketable plants were in plots treated with all rates of AC303630 and MVP (4.67 and 5.8 l/ha). The effectiveness of the thiodicarb + mevinphos mixture was due to mevinphos. The highest rate of MVP, 8.2 l/ha, was less effective than lower rates.

Test 6, 1990

Collard was transplanted on 24 May 1990. Initial experimental design was a strip-plot. Main plots were treatments split in strips of two 15-m long subplots. Subplots were 4-5 and 7-day spray schedules. Insecticides for the 7-day schedules were applied 11 and 18 June. Those for the 4-5-day schedules were applied 11, 15, 21, and 26 June and 2 July. Beginning 21 June the 7-day schedule was discontinued due to poor performance of most of the materials. Experimental design thereafter was a randomized complete block. Safer Insecticidal Concentrate (Safer Inc.,

Newton, MA) was added to Javelin in an attempt to improve performance. Valent X-77 spreader-sticker was used with all materials (except the oil combinations) at a concentration of 0.25%. Rainfall and irrigation during the test period totaled 90 mm.

The DBM and the CL were the principal insect pests on 5 July (Table 6). Fewest DBM, lowest damage scores and most marketable plants were in plots treated with MVP (4.47 l/ha with and without Safer Ultrafine oil and 2% Safers Concentrate) and teflubenzuron (.045 kg AI/ha), Javelin (1.12 kg/ha with 1% Safer Concentrate), Cutlass E S (4.67 l/ha) and Cutlass

Table 5. DBM damage scores and marketability of cabbages treated[a] with insecticides. Test 5, 1990.

Treatment	Liters or kg AI/ha	Avg.[b] DBM	Damage[c] score	% Marketable[d]
Cutlass WP	2.12	15.4	3.02	10.0
Javelin WG	1.12	12.1	2.35	42.5
Javelin WG	0.8	12.2	2.82	20.0
Esfenvalerate 0.66 EC	0.06	34.3	3.50	7.5
Thiodicarb 3.2 E	0.7	32.6	3.65	0.0
Thiodicarb 3.2 E	0.9	28.2	3.65	5.0
Thiod. + Dipel 2X W	0.7 + 1.12	21.2	2.90	35.0
Larv. + Javelin WG	0.7 + 1.12	17.4	2.88	32.5
Thiod. + mevinphos[d]	0.7 + 1.12	5.2	1.92	100.0
AC303630 SC	0.07	4.4	2.40	75.0
AC303630 SC	0.11	0.3	2.10	95.0
AC303630 SC	0.22	1.5	1.75	100.0
AC303630 SC	0.34	1.2	2.15	95.0
MVP	3.5	4.4	2.70	72.5
MVP + Ultrafine oil	3.5 + 4.7	6.4	2.22	72.5
MVP	4.67	4.7	2.42	75.0
MVP	5.8	2.5	2.15	85.0
MVP	7.0	3.9	2.58	67.5
MVP	8.2	3.4	2.30	37.5
Untreated check		34.4	5.00	0.0
LSD (P = 0.05)		7.6	0.56	24.0

[a]Treated 23 April, 1, 10, 16, 23 May. [b]30 April, 7, 14, 27 May. [c]Score, 0-5 scale: 0 = no, 1-2 = light to moderate to outer leaves; 3-5 = light to severe to head. [d]Marketable = heads with 0-2 scores.

Table 6. DBM and CL feeding damage and market quality on collards treated[a] with insecticides. Tifton, Georgia, Test 6, 1990.

Treatment	Liters or kg/ha	No./5 Plants		Damage score[b]	% Marketable
		DBM	CL		
MVP + 1% Safer Conc.	4.47	7.0	16.2	1.9	82.5
MVP + 2% Safer Conc.	4.47	2.2	10.0	1.9	92.5
MVP + 1% Safer UF Oil	4.47	1.0	5.2	1.9	92.5
MVP + 0.5% Safer UF Oil	4.47	3.2	9.2	1.7	92.5
MVP	4.47	3.0	4.8	2.2	77.5
Javelin WG + 1% Safer Conc.	1.12	7.2	13.2	2.4	52.5
Cutlass WP	2.24	6.5	9.2	3.1	32.5
Teflubenzuron 5E[d]	0.045	4.2	16.0	1.9	85.0
AC303630 IEC	0.22	0.5	0.2	0.8	100.0
Untreated check		12.2	45.0	5.0	0.0
LSD (P = 0.05)		4.6	9.8	0.5	23.0

[a]Treated 11, 15, 18, 26 June and 2 July. [b]Score, 0-5 damage scale: 0 = no, 1-2 = light to moderate to outer leaves, 3-5 = light to severe to head and wrapper leaves. [c]Plants with scores of 0 to 3. [d]Applied 11 and 18 June at .015 kg AI/ha, 26 June and 2 July at 0.045 kg AI/ha. No application on 15 June.

WP (2.2 kg/ha) were not significantly different from the check. All treatments gave significant reduction of the CL.

Discussion

The pyrethroid insecticides, esfenvalerate, bifenthrin and cyhalothrin were generally incapable of maintaining marketable cabbages when the DBM was one of the principal defoliators (Tables 1, 3 and 5) and resistance to insecticides was apparent. Application of permethrin, although producing mostly marketable heads in 1988, was ineffective without a synergist in 1989 (Table 4). Where the DBM was not a major factor (Table 3), permethrin was effective at twice the recommended rate (0.22 kg AI/ha). Esfenvalerate was also effective but at a high rate (0.06 kg AI/ha, Table 2). In Texas, Magoro and Edelson (1990) also reported pyrethroid resistance in the DBM but that full-recommended rates of pyrethroids were still effective. With continued use of these pyrethroids, it is unlikely that even high rates will be effective against the DBM. Mevinphos was effective as a component in a mixture with thiodicarb or Dipel against the DBM (Tables 1, 5) and would have been as effective without thiodicarb. It is used by many Georgia growers.

The four microbial insecticides Dipel, Javelin, Cutlass and MVP gave somewhat inconsistent results. In Tests 1 and 2 (Tables 1, 2) weekly applications of 1.2 l/ha of Dipel ES, 0.56 kg/ha of Dipel 2X WP, and high rates of Cutlass WP and Cutlass OF were capable of producing greater than 95% marketability, based on less strict standards. No one of these treatments gave more than 60% of damage-free best heads (scores of 2 or less). Combinations of Dipel with mevinphos, esfenvalerate, methomyl and fenoxycarb gave some improvement. Effective protection by microbials against CL and imported cabbage worm in the northern states and Canada was reported by Shelton et al. (1982), Sears et al. (1983), Tompkins et al. (1986) and Jaques (1988). These tests and Georgia Tests 1 and 2 were performed when temperatures were moderate and pest pressure was not excessive. During mid summer in 1989 on collards (Test 3, Table 3), temperatures were hot, pest pressure was high and rainfall was excessive (318 mm rainfall in June alone). None of the four *B. thuringiensis* formulations gave sufficient protection. Similarly, in 1990 on collard, weekly applications of all materials had to be discontinued in favor of a 4-5 day schedule. During late 1989 (Table 4) these same materials gave adequate protection with infrequent applications.

The molting inhibitor, flucycloxuron, was moderately effective against the DBM in 1988 (Table 1). In 1989 (Table 3), when used during the initial two applications when the DBM was the principal pest, it was necessary to substitute esfenvalerate or permethrin (Table 3) when the CL population began to increase in these treatment plots. Another class of molting inhibitor, teflubenzuron, similarly gave better control of the DBM than the CL on collard, although damage scores were among the lowest of all materials tested. Jansson and Lecrone (1988) also obtained excellent control against resistant DBM in southern Florida. A new compound, AC303630, was effective against both species (Tables 2 and 5) and shows promise for use in a resistance management program.

Before 1987, in Georgia and elsewhere when the pyrethroid insecticides were effective and the CL was the key pest of cole crops, insecticidal applications on cabbage could be delayed until heads began to form, and thereafter thresholds were 0.5 CL equivalents for New York (Shelton et al. 1982), 1-2 holes/plant (Chalfant et al. 1979) , 0.1-0.3 larvae/plant (Chalfant 1979). Shelton et al.(1982), Sears et al. (1983) concluded that action thresholds for the microbials would have to be lower than those used by the chemical pesticides. Applications will also have to be more frequent because of rapid degradation of the microbials and greater reproductive potential of the DBM. Frequent applications of insecticides is a recipe for development of resistance of insecticides (Georghiou 1983) including *B. thuringiensis* (Tabashnik 1990) and resistance management strategies must be in place.

Resistance management is complicated by simultaneous occurrence of multiple species which may require insecticidal mixtures. *Bacillus thuringiensis* was generally more effective against the DBM than the CL in the Georgia experiments. Permethrin and esfenvalerate were most effective against the CL. Although piperonyl butoxide increased efficacy of permethrin against the DBM in 1989 (Table 4), the DBM is capable of developing resistance to this synergist (Chen and Sun 1986). Furthermore, pyrethroids are much less responsive to synergism once the DBM has already developed pyrethroid resistance (Sun et al. 1986).

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Integration of an Insect Growth Regulator and *Bacillus thuringiensis* for Control of Diamondback Moth

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Abstract

Studies conducted between 1987 and 1990 evaluated the effectiveness of a benzoyl phenyl urea-based insect growth regulator, teflubenzuron, *Bacillus thuringiensis* var. *kurstaki*, and a combination of these approaches at managing diamondback moth (DBM) (*Plutella xylostella* (L.)) populations on cabbage in southern Florida. Between 3 and 10 applications of teflubenzuron were effective in reducing DBM populations and protecting heads from damage during the first 2 years. A strategy that limited the number of teflubenzuron applications to ≤6 and involved weekly applications until heading and biweekly applications thereafter was most effective at reducing DBM populations. Efficacy improved when this approach was integrated with *B. thuringiensis*. Results from the 4 years, however, showed that the efficacy of teflubenzuron decreased over time. Concomitant failure of *B. thuringiensis*-based products applied at weekly intervals was also observed during the last year. These results suggest that DBM populations in southern Florida might be developing resistance to these nonconventional insecticides.

Introduction

Cabbage, *Brassica oleracea* var. *capitata* L., is an important vegetable crop in Florida and the Caribbean. In 1984-85, over 7000 acres (2880 ha) valued at over US$50 million was planted in Florida (Anon. 1986). The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), is a cosmopolitan insect pest of brassica crops, and the most important pest of cabbage and other crucifers worldwide (Talekar and Griggs 1986). It is the most important insect pest of cabbage in Florida (Jansson and Lecrone 1988) and most of the Caribbean (Salinas 1986). Talekar et al. (1985) listed over 1000 publications on this pest through 1984. This pest has developed resistance to most conventional chemical insecticides in most parts of the world (Sudderuddin and Kok 1978; Sun et al. 1978; Liu et al. 1981, 1982; Georghiou 1981; Cheng 1986; Miyata et al. 1982, 1986; Chen and Sun 1986; Sun et al. 1986; Tabashnik et al. 1987; Tabashnik and Cushing 1989; Kao et al. 1989; Magaro and Edelson 1990; Wyman and Shelton, in press) and some nonconventional insecticides such as *Bacillus thuringiensis* (Tabashnik et al. 1990; Wyman and Shelton, in press). Insecticide-resistant DBM populations are also found in central (G. L. Leibee, University of Florida, unpublished data) and southern Florida (R. K. Jansson, University of Florida, unpublished data). A recent study showed that considerable variation in insecticide resistance was found among 40 populations of DBM in the U.S. (Wyman and Shelton, in press). A polygenic basis for resistance has been suggested (Liu et al. 1981; Tabashnik and Cushing 1989). Tabashnik et al. (1987) showed that considerable intraisland variation in resistance occurred among populations in Hawaii; however, interisland resistance

levels did not differ. Conditions that enhance the development of insecticide resistance, such as high temperatures, long growing season, multiple insect generations, and intense insecticide pressure (Sun et al. 1978), are present in each of these areas where resistance has developed.

The benzoyl phenyl urea chitinase compounds (e.g. teflubenzuron) have potential for managing DBM populations (Jansson and Lecrone 1988). This group of compounds has been evaluated for its potential at managing insecticide-resistant DBM populations (Becker 1986; Kohyama 1986; Lim and Khoo 1986; Sagenmueller and Rose 1986; Perng and Sun 1987; Jansson and Lecrone 1988); however, certain populations of DBM in Southeast Asia have developed resistance to teflubenzuron (Perng et al. 1988). The present studies were conducted to evaluate the potential of teflubenzuron for controlling DBM and to develop a resistance-management program for this compound in southern Florida by integrating this compound with conventional and genetically improved *B. thuringiensis* products.

Materials and Methods

Experiments were conducted between 1987 and 1990. All experiments were conducted in a Rockdale soil that was fumigated with Terr-O-Gas (75% methyl bromide, 25% chloropicrin; 242 kg/ha) and covered with black (1987), blue (1988), or white on black (1989 and 1990) plastic mulch about 2 weeks before planting. Mulch was perforated 1-5 days before planting. Certified seeds of 'Rio Verde' cabbage were incorporated into a germination mix (Pro-Mix) direct-seeded, and spaced 0.3 m apart within each of two rows per bed that were 0.76 m apart on 1.83-m center beds. All experiments followed standard production practices for cabbage in Florida (see Jansson and Lecrone 1988). In 1987 and 1988, plants were sprinkler-irrigated twice a week (4.7-6.3 cm/ha/irrigation). In 1989 and 1990, plants were irrigated 4 hours/day using a turbo T-tape drip irrigation system (model 40) (5.0 l/m/hour).

Experiments were arranged as randomized complete block designs with four replications. In 1987, treatment plots were 2 rows (1 bed) wide by 10.7 m long. A 1.5 m buffer of nontreated plants separated treatment plots. In the remaining 3 years, treatment plots were 4 rows (2 beds) wide by 12.2 m long. A 3 m buffer of nontreated plants separated each replicate and one nontreated bed separated treatment plots within replicates.

Efficacy and residual activity of teflubenzuron

In the first 2 years, the efficacy and residual activity (efficacy) of teflubenzuron was determined. Complete details of these studies have been reported previously (Jansson and Lecrone 1988). In 1987, six treatments were evaluated for their efficacy against DBM: weekly applications of teflubenzuron (0.022 and 0.044 kg AI/ha); methomyl (0.5 kg AI/ha) in combination with conventional *B. thuringiensis* var. *kurstaki* (Dipel 1X; 0.28 kg/ha); methamidophos (0.56 kg AI/ha); fenvalerate (0.11 kg AI/ha); and a nontreated check. In 1988, different application intervals of teflubenzuron were evaluated and compared to conventional management approaches. Treatments included applications of teflubenzuron (0.033 kg AI/ha) at 1-, 2-, and 3-week intervals, weekly applications of fenvalerate (0.11 kg AI/ha), weekly applications of methomyl (0.5 kg AI/ha) in combination with *B. thuringiensis* var. *kurstaki* (Dipel 2X; 0.28 kg/ha), and a nontreated check. Treatments were applied with a tractor-mounted single bed boom sprayer with two disc cone nozzles (D-4, no. 24) on each side of the bed and one nozzle over the center of each bed. The sprayer delivered 934.6 l/ha at 4.8 km/hour.

Development of an application strategy for teflubenzuron

Various application strategies of teflubenzuron were compared during the following 2 years to develop a program for teflubenzuron that would maximize insect management and minimize the number of teflubenzuron applications ($\leq$ 6 per season). Three treatment strategies were

compared in 1989: weekly applications of teflubenzuron (0.033 kg AI/ha) until heading and then at 2-week intervals (biweekly) thereafter; applications made biweekly until heading and then judiciously thereafter; and applications made before heading, at heading, and 3 weeks after heading. These treatments were compared with applications of teflubenzuron (0.033 kg AI/ha) in combination with a conventional *B. thuringiensis* var. *kurstaki* (Dipel 2X) (0.56 kg/ha) made biweekly until heading and then judiciously thereafter, weekly applications of methomyl (1.0 kg AI/ha) in combination with Dipel 2X (0.56 kg/ha), and nontreated plants. Spraying was done as described previously.

In 1990, two application strategies of teflubenzuron (0.033 kg AI/ha) were compared: applications made weekly until heading and biweekly thereafter; and those made biweekly until heading and judiciously thereafter. Each of these strategies was evaluated alone and in combination with a transconjugated strain of *B. thuringiensis* var. *kurstaki* (Cutlass WP) (2.24 kg AI/ha). These four treatments were compared with biweekly applications of teflubenzuron, weekly applications of mevinphos (0.56 kg AI/ha) in combination with Dipel 2X (0.56 kg/ha), and nontreated plants. Mevinphos was chosen as the chemical standard in this experiment because methomyl was no longer effective at managing DBM populations.

Integration of teflubenzuron with *B. thuringiensis*

An additional study was conducted in 1990 to determine the effects of various combinations and alternating application strategies for teflubenzuron and *B. thuringiensis* on population reduction and field plant protection. The conventional *B. thuringiensis* product, Dipel 2X, was compared with Cutlass, for crop protection when integrated with teflubenzuron. Nine treatments were evaluated: teflubenzuron (0.033 kg AI/ha) in combination with Dipel 2X (0.56 kg/ha) or Cutlass WP (2.24 kg AI/ha) rotated on alternate weeks with the corresponding *B. thuringiensis* product alone; combinations of teflubenzuron and *B. thuringiensis* applied biweekly without alternate week rotations; weekly applications of Cutlass WP (2.24 kg AI/ha); weekly applications of Dipel 2X (0.56 kg/ha); biweekly applications of teflubenzuron (0.033 kg AI/ha); weekly applications of mevinphos (0.56 kg AI/ha) in combination with Dipel 2X (0.56 kg/ha); and a nontreated check.

Sampling and data analysis

In all experiments, the numbers of small, medium, and large DBM larvae and pupae were counted on 8 plants per treatment plot, 32 plants per treatment per sample date. With few exceptions, the fields were monitored at weekly intervals throughout each season. In 1987, data were collected from the center 6 m of each treatment plot; in the remaining 3 years, only the center 9 m of the two middle rows of each treatment plot were sampled. Foliar damage was rated visually on 24 plants per treatment plot at harvest using a scale from 1 to 6 as follows: 1, no apparent insect feeding; 2, minor insect feeding on wrapper and outer leaves, 0 to 1% leaf area eaten; 3, moderate insect feeding on wrapper or outer leaves with no head damage, 2 to 5% leaf area eaten; 4, moderate insect feeding on wrapper or outer leaves with minor feeding on head, 6-10% leaf area eaten; 5, moderate to heavy feeding on wrapper and head leaves and a moderate number of feeding scars on head, 11-30% leaf area eaten; and 6, considerable insect feeding on wrapper and head leaves with head having numerous feeding scars, over 30% of leaf area eaten (Greene et al. 1969). The percentage of marketable heads was determined by calculating the percentage of heads with ratings ≤ 3.

Data were analyzed using least squares analysis of variance (Neter and Wasserman 1974). Numbers of DBM per plant and percentage of marketable heads were transformed to $ln\ (X + 1)$ and to the arcsine, respectively, to stabilize error variance. The Waller-Duncan K-ratio t-test was used to separate treatment means (Waller and Duncan 1969).

Results and Discussion

Efficacy and residual activity of teflubenzuron

Abundance of DBM on cabbage did not differ among most treatments early in the growing season in 1987; however, differences in efficacy were more apparent as the season progressed (Table 1). By the latter part of the season, results showed that teflubenzuron was consistently most efficacious (although not consistently significant) at reducing DBM populations. Similar results were found at harvest. Plants treated with teflubenzuron (either 0.022 or 0.044 kg AI/ha) produced greater percentages of marketable heads than all other treatments (Table 1). Because the two rates of teflubenzuron resulted in comparable levels of control and field plant protection, an intermediate rate of teflubenzuron (0.033 kg AI/ha) was used in subsequent experiments.

In 1988, fewer numbers of DBM larvae were found on plants treated with teflubenzuron than on plants treated with other insecticides and on nontreated plants (Table 1). On most dates, abundance of DBM larvae did not differ among plants treated with teflubenzuron at 1-, 2-, and 3-week intervals. Adequate control of DBM was achieved with as few as three and five applications of teflubenzuron. Less than adequate control was obtained on plants treated with 10 applications of methomyl in combination with Dipel 2X and those treated with 10 applications of fenvalerate. Results were similar when each size category of larvae and pupae was analyzed separately (see Jansson and Lecrone 1988). Similar results were found at harvest. The greatest percentage of marketable heads was found on plants treated with weekly applications of teflubenzuron followed in decreasing order by plants treated with teflubenzuron at 2- and 3-week intervals, weekly applications of methomyl in combination with Dipel 2X, weekly applications of fenvalerate, and nontreated plants (Table 1).

Development of an application strategy for teflubenzuron

In 1989 and 1990, population pressure of DBM was considerably higher than in the two previous years. This might have been due, in part, to the use of drip irrigation in the latter two years, overhead irrigation was used in the former 2 years. Several authors showed that overhead irrigation reduced population density of DBM (Nakahara et al. 1986; Talekar et al. 1986; Tabashnik and Mau 1986).

Abundance of DBM on cabbage plants was affected by the application strategy of teflubenzuron use. DBM larvae were least abundant on plants treated with teflubenzuron weekly until heading and then biweekly thereafter (Table 2). Comparable levels of control were achieved on most dates when teflubenzuron was applied alone at 2-week intervals until heading and then judiciously thereafter and when the same strategy was applied in combination with Dipel 2X. Unlike the previous year, three applications of teflubenzuron (one before heading, one at heading, and one 3 weeks after heading) resulted in poor control of DBM.

Plants treated with teflubenzuron in combination with Dipel 2X at biweekly intervals until heading and then judiciously thereafter produced the highest percentage of marketable heads (Table 2). Comparable percentages of marketable heads were produced by plants treated with weekly applications of teflubenzuron until heading and biweekly applications thereafter, and those treated with weekly applications of methomyl in combination with conventional *B. thuringiensis* (Dipel 2X). Percentages of marketable heads on the most efficacious teflubenzuron treatment, however, were considerably lower in 1989 (41.2%) than in 1987 and 1988 (97.9% in both years).

In 1990, DBM larvae were least abundant (although not consistently significant) on plants treated with teflubenzuron in combination with Cutlass, weekly until heading and then biweekly thereafter (Table 2). Applications of teflubenzuron, with or without Cutlass, made biweekly until heading and made judiciously thereafter were less efficacious (although not consistently significant) at reducing DBM populations. In general, biweekly applications of teflubenzuron alone were less efficacious than the other teflubenzuron-based treatments.

Table 1. Effect of various insecticide applications on the infestation of cabbage by DBM in 1987 and 1988.[a]

Insecticide treatment	Rate (kg AI/ha)	Mean no. DBM larvae + pupae per plant on Sample dates								Damage rating	Marketable heads (%)
		2 Jan	11 Jan	16 Jan	26 Jan	30 Jan	9 Feb	16 Feb	23 Feb		
1987											
Methomyl +	0.5										
B. thuringiensis (Dipel)	0.28	0.5 a	0.9 a	0.3 b	0.4 b	0.7 c	0.4 cd	0.6 bc	0.1 c	2.2 c	83.3 b
Methamidophos	0.56	0.8 a	0.9 ab	0.5 b	0.8 b	1.1 b	2.4 b	1.2 b	0.9 b	3.1 b	50.0 c
Fenvalerate	0.11	0.7 a	0.6 b	0.4 b	0.9 b	0.8 bc	1.1 c	0.4 c	0.3 bc	2.1 c	80.2 b
Teflubenzuron	0.022	0.6 a	1.0 b	0.5 b	0.1 b	0.2 d	0.1 d	0.1 c	0.0 c	1.2 d	97.9 a
Teflubenzuron	0.044	0.7 a	0.5 b	0.3 b	0.5 b	0.1 d	0.0 d	0.0 c	0.0 c	1.2 d	97.9 a
Nontreated check	–	0.2 a	1.5 a	1.7 a	2.6 a	3.4 a	5.6 a	3.9 a	3.4 a	4.1 a	16.7 d
1988		29 Jan	5 Feb	12 Feb	19 Feb	26 Feb	4 Mar	11 Mar	21 Mar		
Methomyl +	0.5										
B. thuringiensis	0.28	0.2 bc	1.0 b	0.4 b	0.2 b	0.2 c	0.1 c	0.2 c	0.1 bc	2.6 c	88.5 ab
Fenvalerate	0.11	0.2 bc	1.0 b	0.2 bc	0.4 b	1.4 b	2.2 b	3.2 b	0.8 a	3.1 b	78.1 b
Teflubenzuron, 1 week	0.033	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c	1.4 a	97.9 a
Teflubenzuron, 2 week	0.033	0.2 bc	1.0 b	0.0 cd	0.0 bc	0.0 c	0.0 c	0.2 c	0.1 bc	2.6 c	93.7 a
Teflubenzuron, 3 week	0.033	0.3 b	0.4 bc	0.2 b	0.2 bc	0.1 c	0.2 c	0.0 c	0.0 c	2.8 bc	88.5 ab
Nontreated check	–	0.9 a	5.5 a	2.9 a	8.1 a	8.1 a	19.0 a	8.0 a	0.3 b	4.1 a	5.2 c

[a]Means within a column for each year followed by the same letter do not differ among treatments by the Waller-Duncan *K*-ratio *t*-test (Waller and Duncan 1969).

Table 2. Influence of various strategies of teflubenzuron application on the infestation of cabbage by DBM in 1989 and 1990.[a]

Insecticide treatment	Rate (kg AI/ha)	Mean no. DBM larvae + pupae per plant on								Damage rating	Marketable heads (%)
		Sample dates									
		30 Jan	7 Feb	15 Feb	19 Feb	1 Mar	7 Mar	13 Mar	20 Mar		
1989											
Methomyl + Dipel W	1.0 + 0.56	6.9 a	11.4 b	6.4 b	5.5 cd	2.7 b	0.4 a	0.2 a	0.4 a	3.6 cd	36.4 ab
Teflubenzuron W/B	0.033	5.7 a	4.6 c	2.7 c	2.2 e	1.1 bc	0.4 a	0.1 a	0.1 a	3.5 d	39.6 ab
Teflubenzuron B/J	0.033	5.6 a	3.8 c	9.4 b	7.7 c	0.7 c	0.3 a	0.0 a	0.0 a	3.7 bc	28.1 b
Teflubenzuron BH/H/T	0.033	4.3 a	3.7 c	8.1 b	23.1 b	8.3 a	0.4 a	0.2 a	0.2 a	3.9 b	15.6 c
Teflubenzuron B/J + Dipel	0.033 + 0.56	6.2 a	5.1 c	6.4 b	3.7 de	1.0 bc	0.3 a	0.1 a	0.1 a	3.5 d	41.2 a
Nontreated check	–	5.5 a	25.1 a	78.1 a	111.9 a	7.6 a	0.0 a	0.0 a	0.4 a	5.0 a	0.0 d
1990		6 Mar	14 Mar	26 Mar	3 Apr	10 Apr	16 Apr	23 Apr	30 Apr		
Mevinphos + Dipel W	0.56 + 0.56	2.4 a	1.8 c	20.6 a	15.0 a	23.5 a	7.0 bc	3.5 b	0.8 b	4.4 b	0.0 a
Teflubenzuron W/B	0.033	2.2 a	3.3 bc	11.0 ab	3.0 b	20.1 ab	5.6 c	2.2 c	0.7 b	4.4 b	1.0 a
Teflubenzuron + Cutlass W/B	0.033 + 2.24	2.5 a	2.2 c	8.6 b	3.4 b	8.7 bc	4.1 cd	1.1 d	0.3 b	4.5 b	1.0 a
Teflubenzuron B/J	0.033	2.8 a	5.0 b	19.4 a	3.5 b	4.5 cd	2.8 d	1.3 cd	0.8 b	4.1 c	5.2 a
Teflubenzuron + Cutlass B/J	0.033 + 2.24	1.7 a	1.7 c	25.3 a	4.2 b	2.8 d	2.8 d	1.6 cd	0.8 b	4.1 c	6.3 a
Teflubenzuron B	0.033	2.3 a	4.4 ab	17.9 a	5.3 b	19.2 a	12.2 b	2.1 c	1.1 b	4.5 b	0.0 a
Nontreated check	–	2.6 a	9.1 a	21.4 a	13.3 a	23.4 a	34.5 a	8.0 a	7.4 a	4.8 a	0.0 a

[a]Means within a vertical column for each year followed by the same letter do not differ among treatments by the Waller-Duncan *K*-ratio *t*-test (Waller and Duncan 1969). [b]W, applications made weekly; WB, applications made weekly until heading and then biweekly thereafter; BJ, applications made biweekly until heading and then judisciously thereafter; BH/H/T, one application made before heading, at heading, and three weeks after heading; B, applications made biweekly.

Table 3. Effect of teflubenzuron and for *B. thuringiensis* application on the infestation of cabbage by DBM in 1990.[a]

Insecticide treatment	Rate (kg AI/ha)	Mean no. DBM larvae + pupae per plant on								Damage rating	Marketable heads (%)
		Sample dates									
		5 Mar	16 Mar	26 Mar	4 Apr	10 Apr	16 Apr	23 Apr	30 Apr		
Mevinphos + Dipel W	0.56 + 0.56	1.4 a	11.4 bcd	28.4 bc	9.6 a	17.3 a	5.3 bc	1.1 b	0.4 bc	4.4 a	1.0 a
Teflubenzuron + Cutlass/Cutlass R	0.033 + 2.24	1.6 a	7.6 d	14.7 d	3.1 a	4.6 bc	2.6 c	0.5 b	0.1 c	4.1 b	10.4 a
Teflubenzuron + Cutlass B	0.033 + 2.24	1.6 a	8.8 cd	25.3 bcd	1.8 a	3.8 c	4.2 bc	1.3 ab	0.4 bc	4.1 b	10.4 a
Teflubenzuron + Dipel/Dipel R	0.033 + 0.56	1.2 a	9.9 bcd	23.6 cd	4.7 a	6.0 bc	2.7 c	0.7 b	0.4 bc	4.2 ab	11.5 a
Teflubenzuron + Dipel B	0.033 + 0.56	1.9 a	10.1 bcd	31.0 bc	3.1 a	7.2 b	3.3 bc	0.9 b	0.3 bc	4.1 b	12.5 a
Teflubenzuron B	0.033	1.8 a	13.2 abc	32.2 bc	3.9 a	5.2 bc	4.3 bc	1.7 ab	0.8 b	4.3 ab	6.3 a
Cutlass W	2.24	1.5 a	16.0 ab	30.3 bc	8.0 a	22.2 a	7.2 b	2.3 ab	0.7 bc	4.4 ab	4.2 a
Dipel W	0.56	1.6 a	19.5 ab	37.6 b	12.2 a	20.3 a	8.6 b	1.5 ab	0.8 b	4.3 ab	6.3 a
Nontreated check	–	1.0 a	25.1 a	68.1 a	12.7 a	25.3 a	14.7 a	3.7 a	3.5 a	4.4 a	4.2 a

[a]Means within a column for each year followed by the same letter do not differ among treatments by the Waller-Duncan *K*-ratio *t*-test (Waller and Duncan 1969). [b]W, applications made weekly; R, combination treatment rotated on alternate weeks wtih the *B. thuringiensis* product; B. applications made biweekly.

The percentages of marketable heads did not differ among the treatments (Table 2). As found in 1989, percentages of marketable heads decreased considerably from the previous year suggesting that DBM populations in southern Florida might have developed resistance to teflubenzuron.

Integration of teflubenzuron with *B. thuringiensis*

Fewer (although not significantly) DBM larvae were consistently found when plants were treated with teflubenzuron in combination with Cutlass and rotated on alternate weeks with Cutlass (Table 3). In general, rotating the combinations on alternate weeks with *B. thuringiensis* (Cutlass or Dipel 2X) did not significantly improve control of DBM when compared with biweekly applications of each combination. Plants treated with Cutlass (in combinations, in rotations, or alone) had fewer (although not consistently significant) DBM larvae than those treated with Dipel 2X. Also, better overall control was achieved when teflubenzuron was integrated with *B. thuringiensis* than when weekly applications of *B. thuringiensis* were made alone. An intermediate level of control was achieved when plants were treated with biweekly applications of teflubenzuron. These data suggest that a greater percentage of the population reduction was attributable to teflubenzuron than to *B. thuringiensis*. Although the same *B. thuringiensis*-based products were effective at managing DBM populations in southern Florida in 1989 when applied at weekly intervals (Jansson et al. 1990 a,b; Leibee et al., in review), a failure of four different *B. thuringiensis* products (Dipel 2X, Cutlass, Javelin, and MVP) was observed in another experiment in 1990 in which these products were applied at weekly intervals (Jansson and Lecrone 1990). Field resistance to one *B. thuringiensis* product, Dipel, has been reported in Hawaii (Tabashnik et al., 1990). The high damage ratings and low percentages of marketable heads produced in all treatments suggested that the efficacy of teflubenzuron and *B. thuringiensis* had decreased from the previous year.

Conclusions

Applications of a benzoyl phenyl urea compound, teflubenzuron, provided excellent control of DBM populations during the first 2 years of field testing. Studies designed to develop a resistance management strategy for this material showed that weekly applications of teflubenzuron until heading and biweekly applications thereafter provided the best control of DBM; however, efficacy of teflubenzuron decreased in each of the following 2 years. Integration of teflubenzuron with *B. thuringiensis* improved control of DBM; however, most of the control was attributable to teflubenzuron, albeit at reduced levels. Decreasing efficacy of teflubenzuron over 4 years and a universal failure of *B. thuringiensis*-based products in 1990 suggested that populations of DBM in southern Florida might be developing resistance to these two nonconventional insecticides. Work is currently underway to confirm this belief.

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18

Effectiveness of Dead-Spore *Bacillus thuringiensis* Formulation Against Diamondback Moth

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Abstract

We developed a process of producing a dead-spore *Bacillus thuringiensis* Berliner var. *kurstaki* HD-1 formulation, Toarow CT, and studied several aspects to improve the productivity of crystal toxin and the insecticidal potency after sterilization to kill spores. The production of cell mass or crystal toxin was highly influenced by aeration and agitation of the culture. The culture broth, within 24 hours after the release of 90% of spores from sporangia, was suitable for formulation into commercial product. We tested a live-spore (Toarow) and a dead-spore (Toarow CT) formulations, both having the similar insecticidal potency, against silkworm. Both formulations were equally effective against diamondback moth, *Plutella xylostella* (L.) when tested by a leaf dip method. Also no differences in mortality and control ratio were observed in a field test either. The dead-spore formulation was thus satisfactory for the control of diamondback moth.

Introduction

Various strains of *Bacillus thuringiensis* Berliner (*Bt*) produce crystal toxins comprised of bipyramidal, cuboidal and irregular proteins. Some of the crystal toxins show selective toxicity to larvae of moths and butterflies and others show insecticidal activity against larvae of flies and mosquitos or show selective toxicity only to beetles represented by Colorado potato beetle (Himeno 1989). *Bt* strains have been classified according to flagellar antigens in addition to pathogenicity (De Barjac and Frachon 1990). *Bt* produces spores almost simultaneously with formation of crystal toxins. Therefore, *Bt* formulations contain both crystal toxins (active ingredient) and spores. The crystal toxins have a highly selective insecticidal effect against the target insect(s), while being harmless to humans, animals, birds and fish. On the other hand, spores are known to have no insecticidal effect (Angus 1954). We developed a technology to sterilize vegetative cells and spores of *Bt* with minimum loss of insecticidal activity of crystal toxins, and created a novel *Bt* insecticide, dead-spore *Bt* formulation (Japanese Pat. No. 831099). The formulation under the name of Toarow CT wettable powder was approved for use in insect control in 1981 (Reg. No. 14459) by the Ministry of Agriculture, Forestry and Fisheries of Japan. In this paper, we will present a number of findings in developing the process to manufacture the dead-spore *Bt* formulation and its effects against diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae).

Materials and Methods

Manufacturing process

The process of manufacturing a dead-spore *Bt* formulation (Toarow CT) is shown schematically in Fig. 1. The strain used is *B. thuringiensis* var. *kurstaki* HD-1, donated

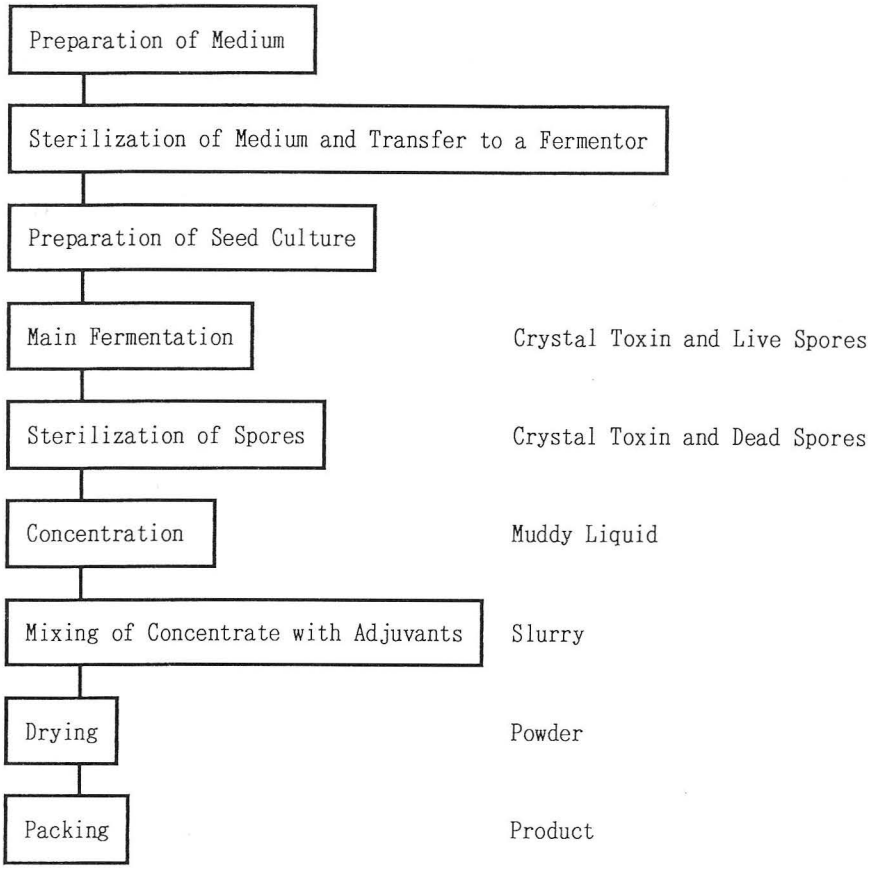

Fig. 1. Flow diagram of manufacturing process of Toarow CT wettable powder (a dead-spore *Bt* formulation).

by Dulmage (Dulmage 1970; De Barjac and Le Mille 1970). This strain has both bipyramidal insecticidal crystal protein (ICP) with high toxicity for lepidopteran larvae and cuboidal ICP with high toxicity for dipteran larvae (Iizuka 1990).

This microbe was cultivated in a conventional submerged fermentor (medium composition: glucose 3%, corn steap liquor 6% and tap water; pH = 7.0 ± 0.1; temp. = 30 ± 1°C). Prior to pilot-scale fermentation, we investigated some factor(s) influencing ICP production by using a 20 l jar-fermentor (Marubishi Co., Type MSJ-205). Agitation speed (rpm) of impeller, flow rate of air (vvm) and working volume were varied. Culture broth was drawn from a jar-fermentor and the precipitate obtained by centrifugation (4°C, 20000g, 10 min) was repeatedly dried in oven and cooled in desiccator until constant weight was obtained. With this value, we calculated a dry cell weight (g/l) of culture broth. The final culture broth was also employed in a bioassay with silkworm (*Bombyx mori* L.). We determined a proper harvesting time by microscopical examination of the culture broth at various stages. The ratios of free spores released from sporangia to total number of spores under five views were employed and the average values of these data were used as the index to judge an end-point of cultivation.

Sterilization of culture broth containing crystal toxins was carried out with a combination of physical treatment (heating, sonication, etc.) and chemical treatment (sterilizers, etc.) (Japanese Patent Publication (Kokoku) No. 51-5047). In industrial scale, sterilization was carried out continuously as follows: the mixture of culture broth and sterilizer was continuously introduced

into the inner part of a double-walled tube, while hot water flowed in reverse through the outer part of it. The number of viable spores and cells was estimated as follows: 1 ml of sample (the sterilized solution or the suspension of product in water, 0.5 g/10 ml) was placed in 10 ml of sterile saline. The number of viable spores (and cells) was determined by counting serial dilutions plated on nutrient agar medium (Difco).

Concentration, mixing of concentrate with adjuvants, drying and packing are carried out successively (Fig. 1) like a conventional insecticide formulation process.

Evaluation of insecticidal potency against silkworm

We adopted a bioassay with silkworm to evaluate ICP's productivity in culture and the two formulations; live spores and dead spores. The bioassay was carried out with third instar larvae of silkworm. A series of 5-6 dilutions of the sample or standard (U.S. standard HD-1-s-1971 of 18000 IU/mg) was added to 10 g of artificial diet (VitaSilk, Kyodo Shiryo), and the mixture was kneaded homogeneously and spread in a petri dish. Twenty silkworm larvae reared on artificial diet were placed on it. The number of dead larvae in each dilution was counted after 3 days of incubation at 28°C. LC_{50} was determined according to Probit Analysis (Finney 1974).

The insecticidal potency (IU/mg) of the sample was calculated as follows:

$$\text{Potency for sample (IU/mg)} = \text{Potency for standard } (= 18000 \text{ IU/mg}) \times \frac{LC_{50} \text{ for standard (ppm)}}{LC_{50} \text{ for sample (ppm)}}$$

The potency of the sample was determined as a mean value from three assays on separate days.

Evaluation of insecticidal potency against DBM

Prior to field trials of Toarow CT against DBM, we carried out several tests in the laboratory. The bioassay was made with first, second and third-instar larvae of DBM in accordance with specific purpose. We used the DBM OSS strain (Virapong et al. 1983) reared in laboratory (16L: 8D and 70-75% RH at 25 ± 1°C). The first, second and third instar larvae corresponded to 4-6, 6-7 and 7-8 days after egg hatch, respectively. A series of 5-7 dilutions of the sample with distilled water containing 0.03% of Triton X-100 was prepared. A cabbage leaf disk (5 × 5 cm) was dipped into 50 ml of each dilution and immediately pulled out and dried in the air at room temperature. Control leaves were dipped in Triton X-100 0.03% only. The dipped leaflet was inserted into a plastic container, at the bottom of which an absorbent paper (Whatman 40) was placed to absorb moisture, and on the top of which a perforated plastic cover was placed for ventilation. Twenty larvae of DBM reared on radish (*Raphanus sativus* var. Osaka 40 nich) seedlings were inserted into each container. The larval mortality was checked after 3 days (1 or 2 days in some cases) of incubation at 25°C. LC_{50} was determined in a manner similar to silkworm.

Field trials were carried out in Obu, Aichi, on cabbage, in which the efficacy of Toarow CT preparation (with dead spores) was compared with that of Toarow preparation (with live spores), under the identical insecticidal potency against silkworm, 10000 IU/mg. Treatments were arranged in a randomized complete block design with two replications using larval density before treatments as the control criterion. The plot was 5 × 15 m and consisted of 100 cabbage plants (at 7-8 weeks after transplanting) in four rows. The formulations were applied as aqueous suspensions at the concentrations of 0.05 and 0.1% with 0.033% of Tokuase (as spreader-sticker, Sankyo Co.). A single application of each preparation was made. One liter was applied for 20 plants per treatment with a manually operated, compressed-air sprayer. An untreated plot was prepared as a control plot. The number of DBM larvae per cabbage plant in each plot were counted before and 3 and 7 days after spray. Mortality and corrected population index based on the change in larval densities were calculated as follows:

$$\text{Mortality } (\%) = (1\ (T_a/T_b)) \times 100$$
$$\text{Corrected population index } (\%) = ((T_a \times C_b)/(T_b \times C_a)) \times 100$$

where 'T' and 'C' show the number of DBM larvae under treatment and control, respectively, and 'b' and 'a' show before and after spray, respectively. These data were also analyzed by analysis of variance. The degree of leaf damage was estimated at 3 or 7 days after the spray.

Evaluation of the effect of UV irradiation on insecticidal potency

Two kinds of experiments were conducted to examine the effect of UV irradiation on the efficacy of *Bt* preparations in the laboratory.

In the first experiment a thin layer of Toarow CT (Lot. 2060P; 10600 ± 670 IU/mg) was exposed to UV irradiation (UV lamp: 15 W). The irradiation time and the distance between a lamp and the sample were varied. One gram of the formulation was mixed with 10 ml of distilled water and 1 ml of the preparation was poured on a petri dish. The sample was dried at room temperature and 5 ml of distilled water was poured into each petri dish after exposure to UV and the insecticidal potency against silkworm was measured by the above method. The residual ratios of potency were obtained.

The second experiment was conducted on cabbage leaves (5 × 5 cm) dipped in the solutions of *Bt* preparation and held under UV irradiation. Samples were the same as those used in the field trials (equivalent to Toarow and Toarow CT). The concentrations of *Bt* solutions were 0 (control), 0.001 and 0.1% all with 0.03% of Triton X-100. The dried cabbage leaves after dipping were placed under the UV lamp at distances of 11 and 22 cm, respectively. After exposure to UV, the leaves were fed on the third-instar larvae of DBM and mortalities were recorded after 3 days.

Results and Discussion

We have developed several processes as shown in Fig. 1 to manufacture a dead-spore *Bt* formulation. *Bt* is an aerobic spore-forming bacteria that can be used to produce cell mass and crystal toxin depending on the media composition in culture (Mummigatti and Raghunathan 1990). We used a simple medium composed of glucose as a carbon source and corn steap liquor as a source of amino acids, minerals and vitamins. The medium was suitable for culturing *Bt*. We therefore investigated the effect of other conditions such as aeration and agitation on the productivity of cell mass and crystal toxin from this microbe.

As shown in Fig. 2, cell mass and insecticidal activity (proportional to the content of ICP) of culture broth increased with the increase of air flow rate and agitation speed, while insecticidal activity of culture broth became maximal at 12 l of working volume. This is supposedly due to inhibitory effect of excessive antifoamer (W = 14 l) in culture and the shortage of volumetric oxygen transfer coefficient, k_La (W = 10 l). Moreover, we scaled up fermentation stepwise to pilot-scale fermentation.

Determination of the endpoint of culture is important to achieve the maximal productivity of crystal toxin. From the trial and error experiments, it was descovered that culture broth within 24 hours after the release of 90% of spores from sporangia is suitable for this purpose.

We compared the efficacy of *Bt* preparation containing dead and live spores (equivalent to Toarow CT and Toarow, respectively) against DBM, both having the same insecticidal potency against silkworm, indoors and in a field. No significant differences in LC_{50} were observed between both preparations when we used cabbage leaf dipping method ($P = 0.01$) (Table 1). No significant differences were observed in mortality and corrected population index under the same dilutions in a field test ($P = 0.05$) (Table 2). In addition, no differences were observed in the degrees of leaf damage between the treatments by two *Bt* preparations.

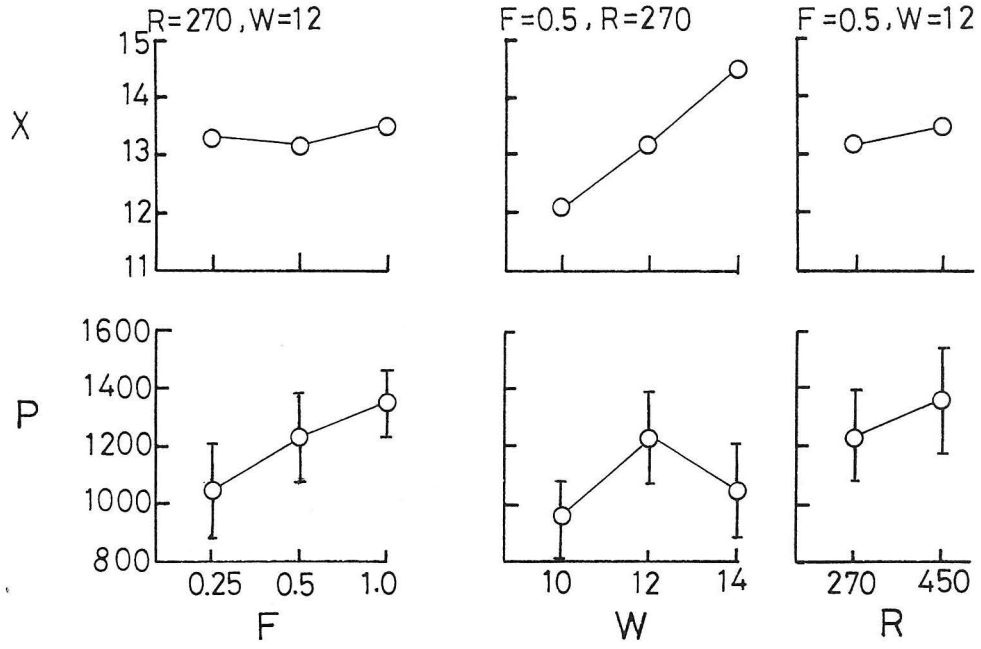

Fig. 2. Effects of aeration, agitation and working volume on productivities of cell mass and ICP in culture of *Bacillus thuringiensis* var *kurstaki* HD-1. Culture conditions are described in text. F, R and W show the flow rate of air (vvm), the agitation speed (rpm) of impeller and working volume (l), respectively. X and P show the maximal value of dry cell weight (g/l) in logarthmic growth phase and insecticidal potency against silkworm (KIU/ml) in finally harvested culture broth, respectively.

Table 1. Comparison of insecticidal potency against DBM between Toarow CT preparation and Toarow preparation with the same insecticidal potency against silkworm in a laboratory test.

Treatment	Insecticidal potency (LC_{50}, ppm) against DBM[a]			
	Exp. 1	Exp. 2	Exp. 3	Mean (±SD)
Toarow CT (dead spore)	19	15	16	17±1.7
Toarow (live spore)	18	16	18	17±0.9

[a]Third-instar larvae of DBM were used and LC_{50} was obtained from the mortality after 3 days. The insecticidal activity of Toarow CT and Toarow against silkworm was adjusted to 10000 IU/mg by the addition of adjuvants.

These results indicate that there is no difference in insecticidal potency against DBM between a dead and a live-spore *Bt* formulation. Similarly, Takaki (1975) reported that both Toarow and Toarow *Bt* (equal to Toarow CT) are effective against mugwort looper (*Ascotis selenaria* Denis et Schiffermüler), which is a pest of tea in Japan.

Table 2. Efficacy of *Bt* preparations containing dead or live spores against DBM in a field test.

Treatment	No. cabbage plants	No. larvae of DBM			Mortality (%)		Corrected population index (%)	
		before spray	3 days after spray	7 days after spray	3 days after spray	7 days after spray	3 days after spray	7 days after spray
Control	20	63	269	729	–	–	–	–
Toarow CT (× 1000)	20	53	8	24	85	55	3.5	3.9
Toarow (× 1000)	20	53	7	28	87	47	3.1	4.6
Toarow CT (× 2000)	20	55	29	138	47	-151	12.3	21.7
Toarow (× 2000)	20	54	43	159	20	-194	18.6	25.4

Mortality (%) and corrected population index (%) were calculated in the same manner as described in text. The mean larval density before spray among the treatments did not vary significantly. The field test was conducted in May 1990, and there was no rainfall during the test.

A gradual decrease of insecticidal activity in the field, due to inactivation of ICP by UV irradiation, is common in all *Bt* formulations. We, therefore, investigated the effect of UV irradiation on the efficacy of *Bt* formulations containing dead or live spores in laboratory tests. First, the relative potency of Toarow CT exposed to UV light under several conditions against silkworm was measured. Table 3 shows that the degree of insecticidal potency diminishes as the distance ('d') between a UV lamp and the sample increases. The half-life of potency was estimated at 9-10 hours at d = 46 cm. Second, we compared the relative potency of a dead-spore *Bt* preparation (Toarow CT) and a live-spore *Bt* preparation (Toarow) against DBM by using the cabbage leaves dipped in the preparations and exposed to UV. As shown in Table 4, the drastic decrease of potency was observed when the irradiation time ('t') was 2 hours. Also, the rate of decrease in insecticidal potency of Toarow CT seems to be less than the one of Toarow.

Ishiguro and Miyazono (1982) demonstrated the fate of viable *Bt* spores on cabbage leaves and a decrease in the pathogenicity to the silkworm in a glasshouse under different exposure conditions. Though the relationship between exposure to UV under the laboratory and the field in our experiment was not confirmed, the relative potency at t = 5 hours and at t = 10 hours (d = 46 cm) in our experiment correspond to the relative potency at t = 3 days and t = 7 days under exposure to UV light in their experiments, respectively. The exposure of cabbage leaves to UV light in our experiment was shortened to suppress chlorosis ($t \leq 2$ hours,

Table 3. Effect of UV irradiation[a] on efficacy of Toarow CT against silkworm in laboratory tests.

Distance (cm)	Relative ratio (%) of residual insecticidal potency against silkworm after hours								
	0	1	3	5	7	10	24	48	72
32	100	87	77	72	79	–	–	–	–
46	100	95	85	75	70	56	48	31	38
53	100	92	81	79	81	–	–	–	–

[a]UV irradiation was conducted as shown in text. [b]Distance between UV lamp and sample (cm).

Table 4. Effect of UV irradiation[a] on efficacy of various concentrations of *Bt* preparation against DBM.

Distance[b]	Mortality (%)[c] of larvae of DBM on treated cabbage leaves														
(cm)	t[d] = 0	0.5	2	0	0.5	2	0	0.5	2	0	0.5	2	0	0.5	2
	Toarow CT 0.001%			Toarow 0.001%			Toarow CT 0.1%			Toarow 0.1%			Contol		
11	47	3	10	20	7	7	93	80	77	97	77	70	10	10	7
	50	13	10	40	13	10	100	90	90	100	100	90	10	10	7
22	47	13	7	20	3	7	93	73	83	97	93	73	7	0	0
	50	47	13	40	43	7	100	100	90	100	100	77	10	0	7

[a]UV irradiation was conducted as shown in text. [b]Distance between UV lamp and sample (cm). [c]Mortality (%) after 2 days in the upper row and after 3 days in the lower row. [d]t: the irradiation time (hours). Thirty 3rd-instar DBM larvae were placed on each cabbage leaf.

d≤22 cm). Our results on the mortality of DBM fed on the cabbage leaves dipped in *Bt* preparations and exposed to UV might suggest that the decrease in the number of spores due to UV irradiation relates to the mortality of DBM in the case of a live-spore *Bt* preparation.

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19

Occurrence of Resistance to *Bacillus thuringiensis* in Diamondback Moth, and Results of Trials for Integrated Control in a Watercress Greenhouse

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Abstract

High levels of resistance to *Bacillus thuringiensis* Berliner in diamondback moth *Plutella xylostella* (L.), (LC_{50} more than 280 ppm) was observed in a watercress greenhouse in Osaka Prefecture in 1988. Populations of egg, larva, pupa and adult of diamondback moth were monitored weekly throughout 1988. Population of the larva fluctuated from nearly 0 to 1,300,000. Three control methods were attempted: Insect Killer (high voltage electric shocker with luring purple color lamps), Konagakon (a sex pheromone dispenser), and the vacuum cleaner to suck adult moths. The percentage of moths killed by 24 sets of Insect Killer in the greenhouse was estimated at less than 15% of the emerged adults even in summer. The percentage of mating reduction by Konagakon depended on moth density (more than 90% of mating reduction if 1 moth/m^2; less than 50% if 3 moths/m^2). The vacuum cleaner reduced moth population by 50% in every operation. It is therefore possible to reduce the moth density where Konagakon is effective through five or six operations of vacuum sucker.

Introduction

Diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) has developed resistance to most organophosphorus and carbamate insecticides used in Japan since 1971. It has also developed resistance to synthetic pyrethroids only 1 or 2 years after these insecticides were marketed in 1983 (Hama 1987). A *Bacillus thuringiensis* Berliner (*Bt*) formulation was also introduced in 1983 in Japan, and its effectiveness on DBM did not decrease for some time, although Morishita and Azuma (1987) reported a population with slightly higher LC_{50} in Wakayama Prefecture. McGaughey (1985) found resistance to *Bt* formulation in *Plodia interpuctella* (Hübner), and so far it is the only report concerning high levels of *Bt* resistance in any lepidopteran insect.

However, a *Bt* formulation (Toarow CT, 7% *Bt* toxin) became less effective in controlling DBM in watercress grown in greenhouses in Osaka Prefecture in 1987. This decrease in susceptibility was confirmed by leaf-dipping method in 1988.

In the present work, the progress of DBM resistance to *Bt* in the greenhouse is reviewed, and the effectiveness of three other control methods discussed.

Cultivation

Watercress was grown in two steel-frame plastic houses (20 m × 50 m × 3 m high), located in a small valley in Kishiwada City in Osaka Prefecture. Hydroponic cultivation of watercress

was started in 1975, and now three people collect and sell 8,000 to 40,000 shoots/day. Watercress is grown throughout the year, and the houses are heated with kerosene heaters from November to March, and the side vinyl of the houses are kept open from April to October. The houses are surrounded by forest and a pond, and the nearest vegetable garden planted to crucifers is at least 300 m from the houses, so immigration of DBM adult moths is assumed to be minimal. DBM is the only insect pest on the watercress, and diseases are not important economically.

Materials and Methods

Resistance to *Bt* formulation

Six tests were done from May 1988 to November 1989. Adult females (30-100 individuals) were collected from the greenhouses on 18 May, 4 and 21 October, 15 November and 13 December 1988 and 16 November 1989, and were given cabbage leaves for oviposition. Hatched larvae were reared on cabbage leaves and the third instar larvae were used for the tests. Susceptibility of Toarow CT was determined by leaf-dipping, i.e. cabbage leaves were dipped in the *Bt* solution for 30 sec, dried in the air and placed into a plastic case (4 cm long, 10 cm diameter). Ten to fifteen larvae were released in each of 2-6 cases, each case being one replicate and dead larvae were counted 3 days later (except in November 1989, 2 days later). Each test consisted of 5-11 concentrations of the *Bt* solution including 70 ppm, and LC_{50} was calculated. All the tests were carried out at 25°C and 12 L:12 D photoperiod except for a test in May 1988 when *Bt* concentration of only 70 ppm was used and the test was carried out at normal room temperature and photoperiod.

Population dynamics

Weekly population densities of egg, larva, pupa and adult were investigated in the greenhouses from January (egg was from March) to December 1988. Forty-eight shoots of watercress longer than 15 cm were systematically chosen in each of the houses, and the numbers of eggs, larvae and pupae on each shoot were counted. Larvae were counted separately as 1st-2nd, 3rd, and 4th instar. Adult density was expressed as the flying number of moths/m^2 by the line transect method, i.e. number of flying moths counted on both sides of 1 m line along a fixed route of 300 m (area 600 m^2) in each of the houses.

Killing moths by electric shocker

Twenty-four sets of high voltage electric shockers with purple color lamps as lures were hung systematically in one of the houses where they were operated from 9 June to 30 September 1988 to kill adult moths. Eight of them were Model YF11990 of Insect Killer (Matsushita Electric Works, Ltd.) and another 16 sets were Model SL-055 (Sun Co. Ltd.). The number of moths killed by each of the electric shockers was counted weekly. Yamada and Kawasaki (1983) showed that the developmental period of larvae and pupae was 9.2 and 3.8 days at 25°C, and the 4th instar larva is assumed to emerge in 6 or 7 days. The number of emerged adults was calculated using the total population of the 4th instar larvae and pupae in the greenhouse, estimated by weekly observations as described earlier, assuming that they (4th instar larva and pupa) emerged in 1 week without any deaths. Killing efficiency (E_K) is then calculated as:

$$E_k(\%) = 100 \times N_k/N_e$$

(N_k: Number of moths killed in a given period)
(N_e: Number of moths emerged in a given period)

Communication disruption by sex pheromone

Sex pheromone of DBM was applied in a pheromone dispenser (Konagakon, Shin'Etsu Chemical Co. Ltd.) for communication disruption of moths in both greenhouses throughout 1988, and its effect was estimated. The 250 m long dispensers covered 0.1 ha in each of the houses, and were replaced every 3 months. Tests for estimating communication disruption were carried out 15 times from May to October 1988, in which 8-30 female adults were collected from the houses and each female placed in a plastic case with cabbage leaves for oviposition. This was done for specific air temperatures and adult flying density (number of flying moths/m^2, as described in the section on population dynamics). If the female laid eggs and the eggs hatched, the female was regarded as mated. If the laid eggs did not hatch, or if the female survived 3 days without laying any eggs, the female was regarded unmated (all tests were carried out at 25°C). The percentage of mating reduction (R_m) was calculated as:

$$R_m(\%) = 100 \times N_u/(N_u + N_m)$$

(N_m: Number of mated females)
(N_u: Number of unmated females)

Elimination of moths by vacuum sucker

Sucking of moths by vacuum cleaner was attempted, and its effect in controlling DBM was estimated. The vacuum pump was an electric single-phase induction motor (Model No. 3 APM of Ebara Co. Ltd., 500 W, capacity: 55 m^3/min) and an accordion-like flexible metal conduit (80 cm long and 20 cm diameter) was attached to it. All the moths sucked by the machine were torn to pieces by the fins of the motor fan.

The vacuum cleaner was operated five times in one-fourth of a greenhouse (250 m^2) on 18 June 1990. The number of moths sucked by the machine was counted, and flying moth density (number of flying moths/m^2) was recorded before every operation and after the last one by line transect method. Weather at the test site was clear and the air temperature was 32°C.

Results and Discussions

Resistance to *Bt* formulation

All cabbage leaves in the tests were extensively eaten, and the percentage of dead larvae at 70 ppm concentration was higher than 50% in two tests (87.5% on 18 May and 53.9% on 21 October 1988), and lower than 50% in the other four tests (Fig. 1) (Tanaka and Kimura 1991). In the latter five tests, LC_{50} was 44 ppm on 21 October 1988, and more than 280 ppm in others. On the other hand, the percentage of dead individuals in two other populations on cabbage in April 1988 in Osaka Prefecture was 100% and cabbage leaves had no feeding holes at the 70 ppm concentration. Morishita and Azuma (1987) reported that LC_{50} of Toarow CT to DBM on cabbage in Wakayama Prefecture was 0.2-0.9 ppm in five populations, and slightly higher (3.9-17.6 ppm) in one population. Therefore, LC_{50} of DBM on watercress in Kishiwada was 20-1000 times higher than that observed by Morishita and Azuma (1987).

Toarow CT was sprayed 15-20 times a year since June 1986 in the houses, and the spraying did not cease even after losing most of its effectiveness, because DBM has developed resistance to all organophosphorus, carbamate, pyrethroid and cartap insecticides. This frequent spraying of Toarow CT, associated with slight immigration of DBM adults, is assumed to cause the resistance in the greenhouses earlier than any other areas.

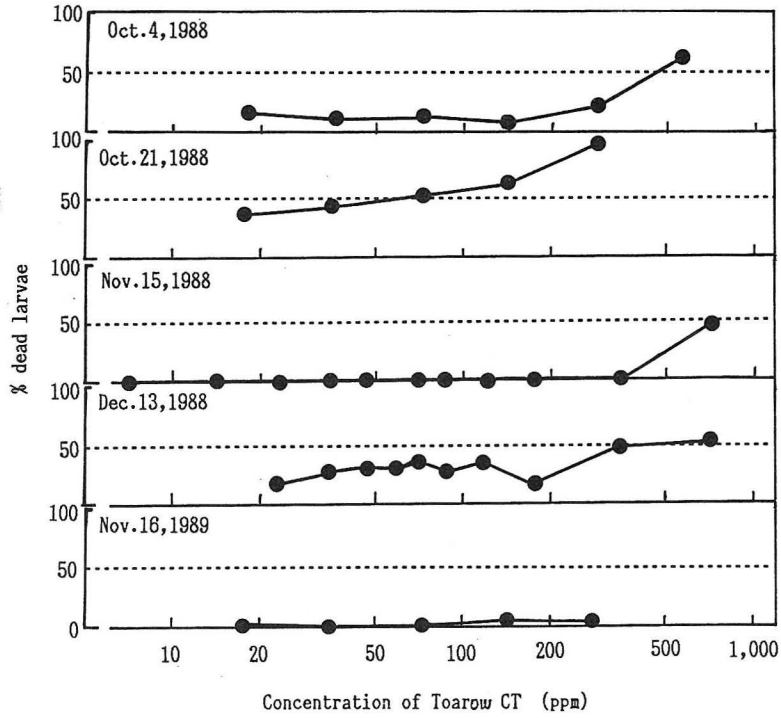

Fig. 1. Susceptibility of 3rd instar larvae of DBM on watercress to *Bt* formulation (Toarow CT) by leaf-dipping method using cabbage leaf. Collection dates of adult moths are shown. (Tanaka and Kimura 1991)

Population dynamics

Population dynamics was quite similar in both houses throughout the year, and the results of a house with a generally higher population are shown in Fig. 2 (Tanaka and Kimura, in press). Population peaks of the flying moth were observed in late March, early May and mid June (10.8 moths/m^2) in the year. The interval between peaks is thought to be the period of one life cycle. Six vague peaks were observed: early July, late July, mid August, early September, late September and early November. Peaks of the eggs, larvae and pupae succeeded those of adults, though they were not so clear from July onwards.

Susceptibility of DBM to Toarow CT decreased markedly in 1987 and 1988 and this formulation is not thought to have been a major factor in suppressing the population in the greenhouses in 1988. Predator and parasitoid wasps of DBM were rarely observed throughout the investigation, possibly because of the occasional spraying of cartap and other pesticides. Disease or other factors may suppress DBM density, though we have not studied this in detail.

Spatial distribution patterns of the eggs and larvae of DBM in the greenhouses were analyzed with Iwao's $\hat{m}$ regression method, and they were both contiguous, i.e., $\alpha = 1.62$ and $\theta = 1.59$ in egg, and $\alpha = 0.07$ and $\beta = 1.60$ in larva (Tanaka and Takahara 1989). The total number of watercress shoots longer than 15 cm was estimated as 680,000 in each of the greenhouses. Short shoots were not suitable for estimating the egg and larval densities, because these stages concentrate on canopy of watercress (Tanaka, unpublished data). Thus the total population of eggs in the greenhouse was estimated to fluctuate from 0 to 6,600,000 $\pm$ 1,800,000 (mean $\pm$ SD), and that of the larva from 0 to 1,300,000 $\pm$ 400,000. Zero in the estimation means undetectable, when the total population was estimated to be under 10,000 in a greenhouse.

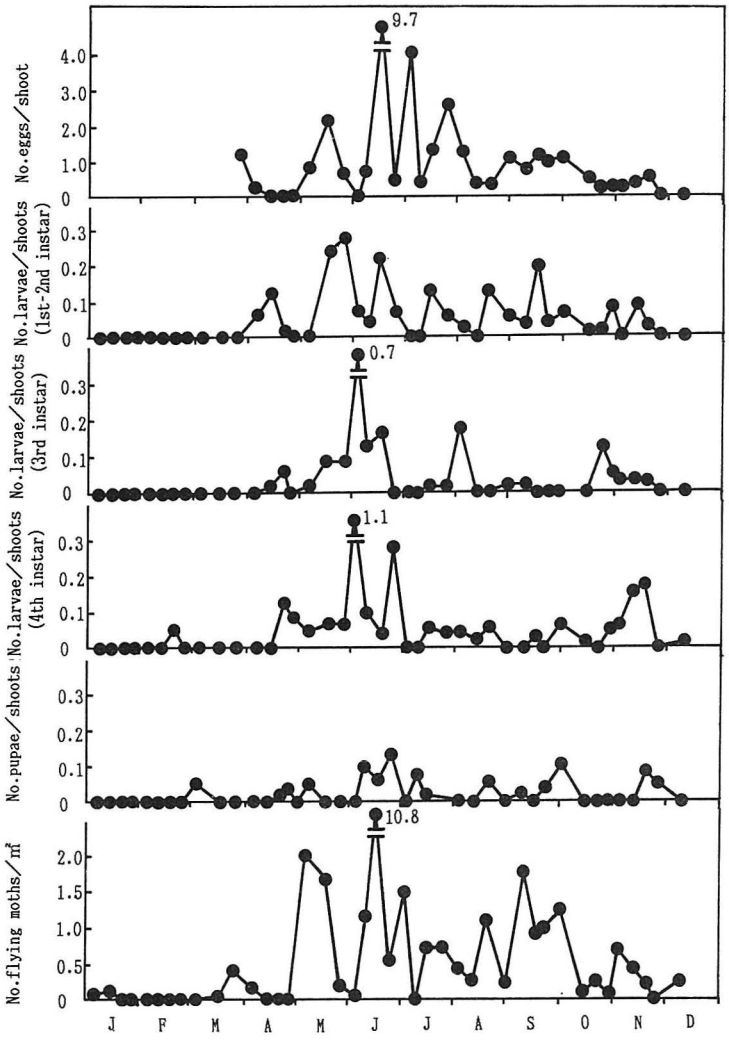

Fig. 2. Population fluctuation of DBM on watercress in 1988; investigation of the egg started in March (Tanaka and Kimura, in press).

Electric shocker

The number of moths emerged, killed, and the killing efficiency are shown in Fig. 3 (Tanaka et al. 1989). The number of emerged adults was 390,000 in July (24 June-24 July), 210,000 in August (25 July-29 Aug.) and 220,000 in September (29 Aug.-30 Sept.), and the killing efficiency was 8.6, 13.6 and 3.7% in the respective months. The total number of emerged adults in 3 months (24 June-30 Sept.) was 820,000 and the average killing efficiency during the period was 8.6%. If the percentage of deaths were 30 or 40%, the estimated values of killing efficiency would increase 43% or 67%. The developmental period of larva and pupa at 20 and 30°C increases by 45% and decreases by 15% respectively, compared with that of 25°C (Yamada and Kawasaki 1983), and killing efficiency decreases by 31% at 20°C and increases by 18% at 30°C compared with 25°C. So the killing efficiency is not high enough to suppress the moth density.

During this test, neither the larval density nor the damage to watercress was suppressed by 24 sets of electric shockers. Moreover, high killing efficiency cannot be expected from October to May, because of low flying activity of moths at night. These results suggest that control of moths with the electric shocker is not practical in the greenhouses.

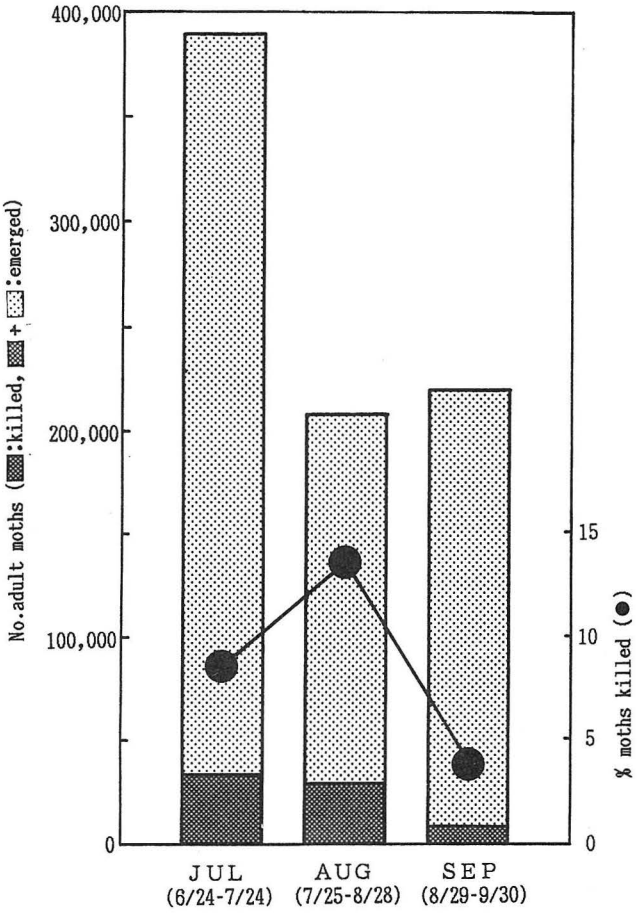

Fig. 3.
Percentage of DBM adults killed by high voltage electric shockers in greenhouse of watercress. (Tanaka et al. 1989).

Communication disruption by sex pheromone

The relation between the flying moth density and the percentage of mating reduction varied depending upon the air temperature (Fig. 4) (Tanaka and Kimura 1990). At temperatures above 20°C, the mating reduction was 100% when the flying moth density (number of flying moths/m^2) was less than 0.1, 50% when the flying moth density was 0.3-0.4 and as low as 10% when the flying moth density was more than 1.0. At temperatures below 20°C, the percentage of mating reduction was less than 30% when the flying moth density was about 0.1, and 0% when the flying moth density was more than 0.2. Estimated mating reduction at each temperature could be drawn as shown by lines in the figure.

Mating reduction achieved in the houses in 1988 can be estimated from the lines in Fig. 4, in association with the fluctuation of the flying moth density as shown in Fig. 2 and the temperature data. The estimated percentage of mating reduction was nearly 100% from January to early March except for mid January (50%), less than 20% from late March to early April, more than 90% in mid and late April, and nearly 0% at the peak of the moth density in May and June.

DBM is not uniformly distributed in the houses, and high adult density is often found at the fringe of the houses, where rather high rates of mating is assumed to occur, even if average adult density in each of the houses is very low.

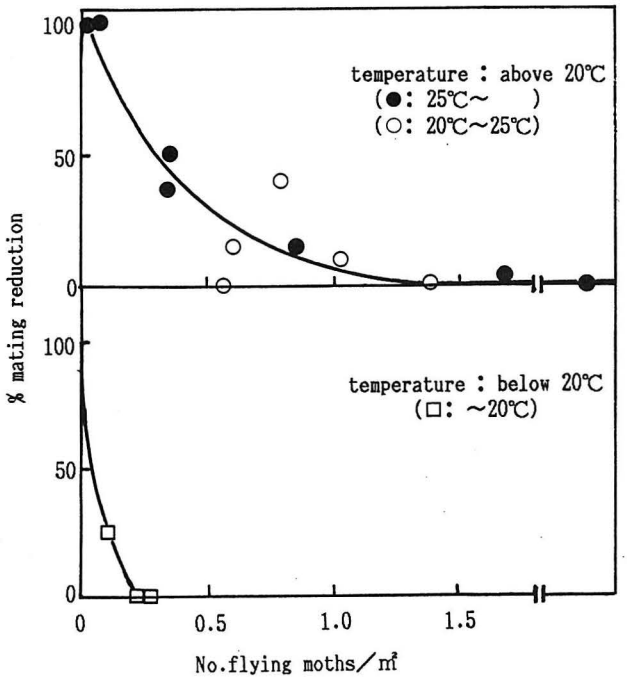

Fig. 4.
Relationship between density of flying moth and percentage of mating reduction by pheromone dispenser in DBM on watercress (Tanaka and Kimura 1990).

Vacuum suction

The flying moth density before the start of the test was 1.78/m^2 and it decreased to 0.91/m^2 after the first operation which sucked 11.07 individuals/m^2 (Fig. 5). The density reduced by about one-half in each operation. The peak of the flying moth density in the houses in summer was 1.0-10.0/m^2 (Fig. 2) and 4-7 operations can reduce the density to less than 0.1/m^2, the level at which the pheromone dispenser effectively reduces the rate of mating.

Since it took 15 min for one operation in one-quarter of a greenhouse in this test, it would take 4-7 hours in a house to reduce the moth density to a level at which the pheromone dispenser is effective. The vacuum cleaner itself was too heavy to be carried on the back for long periods. If the vacuum cleaner is mounted on a rail, for example, and if it takes 20 min for every operation in a house (one-third of the time now required for it), control of DBM using the vacuum cleaner might be practical.

The relation between the accumulated number of eliminated moths (N_e) and the flying moth density (N_f: number of flying moths/m^2) is expressed as a regression line.

$$N_f = -0.083N_e + 1.792 \ (r = -0.9995).$$

The X-intercept of the regression line means the point at which all of the moths are eliminated by the vacuum cleaner, its value (= 21.59) also means the actual number of moths/m^2 at the start of the test. The ratio of the X-intercept to the Y-intercept is 12.05. This is the ratio of the actual moths density to the flying moth density at the start of the test. So the relation between

the flying moth density and the percentage of mating reduction as shown in Fig. 4 can be substituted by another relation, using the actual moth density. A high percentage (more than 90%) of mating reduction then occurs in the actual moth density of less than $1.0/m^2$.

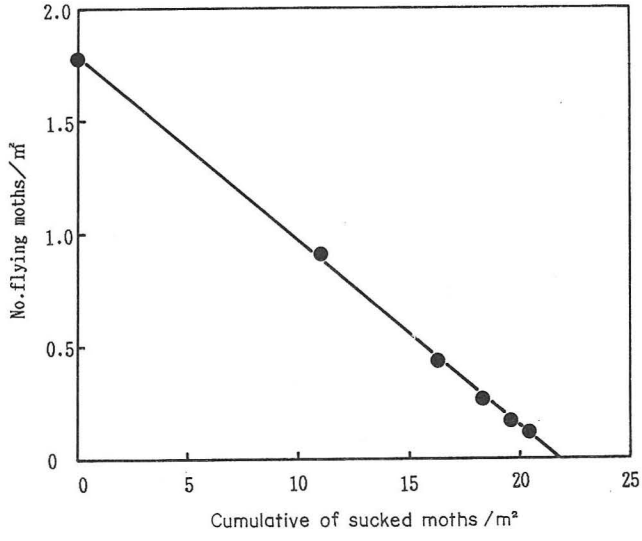

Fig. 5.
Relationship between cumulative number of DBM moths sucked by vacuum cleaner and density of flying moths on watercress.

Conclusions

Growing cruciferous vegetables throughout the year in greenhouses with only chemical control is assumed to create resistance to all insecticides in DBM. So we must seek to develop some physical, biological and cultural control methods, as well as chemical ones, and to estimate the efficiency of each. I investigated the population dynamics and the spatial distribution of DBM in greenhouses of watercress, and estimated the efficiency of three kinds of control methods. The control efficiency of the methods was not satisfactory, though the pheromone dispenser proved to be most effective in situations of low moth density and the vacuum cleaner in situations of high moth density. It is therefore most promising to combine the pheromone dispenser and vacuum cleaner. There is certainly room for improvement in the efficiency of the vacuum cleaner, and the combination may overcome the DBM problem in the greenhouses in the near future. It is important to consider the cost of the control methods. The dispenser-sucker combination is not too costly in the cultivation of watercress, so it could possibly be adopted. Nakahara et al. (1986) reported that DBM in watercress in Hawaii was controlled by use of an intermittent overhead sprinkler system, which disturbed the mating and egg-laying activities of the moths. However the system was not adopted for watercress in Osaka because the sprinkler spraying would dilute the nutrient concentration in hyrdoponics.

Acknowledgments

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Diamondback Moth Resistance to *Bacillus thuringiensis* in Hawaii

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Abstract

Resistance to the microbial insecticide *Bacillus thuringiensis* Berliner has evolved in field populations of diamondback moth, *Plutella xylostella* (L.), in Hawaii. Three field populations that had been treated repeatedly with commercial formulations of the spore-crystal protein complex of *B. thuringiensis* subsp. *kurstaki* developed significant resistance to it. The LC_{50} of the most resistant population was 25-36 times greater than the LC_{50}s of susceptible laboratory strains and 41 times greater than the LC_{50} of the most susceptible field population. Laboratory selection of three strains established from the most resistant field population caused 15-30-fold increases in LC_{50} in nine generations, resulting in 430-820-fold resistance compared with a susceptible laboratory strain. Resistance declined slowly in the absence of treatments. Development of resistance to a commercial formulation containing a mixture of *B. thuringiensis* toxins by field populations of DBM casts doubt on the ability of mixtures to retard evolution of resistance. Slow restoration of susceptibility in the absence of treatments suggests that rotations may not be especially effective for retarding resistance development. We urge judicious use of *B. thuringiensis* to conserve its efficacy.

Introduction

Throughout the world, pests are becoming resistant to pesticides at an alarming rate (NRC 1986; Roush and Tabashnik 1990). The success of conventional pesticides is also threatened by increasing awareness of their toxicity to natural enemies (Johnson and Tabashnik 1991) and their harmful effects on the environment. Microbial insecticides are a promising alternative. The most widely used microbial insecticide, *Bacillus thuringiensis* Berliner (*Bt*), is highly toxic to certain pests, yet it has little or no adverse effect on most nontarget organisms, including humans (Flexner et al. 1986; Wilcox et al. 1986).

Bt is especially useful for control of diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), a worldwide pest of cruciferous vegetables. *Bt* does not harm the hymenopterous parasitoids of DBM (Brunner and Stevens 1986), but it is highly effective against DBM that are resistant to conventional insecticides (Sun et al. 1986). In two independent laboratory experiments, selection with *Bt* for 10 and 30 generations did not increase resistance to *Bt* (Devriendt and Martouret 1976; Krieg and Langenbruch 1981). These laboratory results and the lack of well-documented cases of resistance from field populations led some scientists to conclude that DBM was unlikely to develop resistance to *Bt* under field conditions (e.g., see p. 238 in Talekar and Griggs 1986). However, at the First International Workshop on Diamondback Moth in 1985, C. N. Sun suggested that 'if *Bt* were used on a scale comparable to that of the synthetic insecticides, DBM might become just as resistant' (p. 398 in Talekar

and Griggs 1986). Kirsch and Schmutterer (1988) reported low efficacy of *Bt* against DBM that was suggestive of resistance, but they could not exclude alternative explanations.

As part of a long-term study of insecticide resistance in DBM (Tabashnik 1986; Tabashnik et al. 1987; Tabashnik and Cushing 1989), we measured susceptibility to *Bt* in 1986-87 and 1989-90 for several populations in Hawaii. Based on these surveys, we documented, for the first time, evolution of resistance to *Bt* in open field populations (Tabashnik et al. 1990). In this paper, we review the results of our field studies and subsequent experiments that characterized the response of resistant populations to continuation and relaxation of selection (Tabashnik et al. 1991).

Methods

Field surveys: During 1986-87 we sampled 50-300 individuals from each of six field populations in Hawaii (Fig. 1) (Tabashnik et al. 1990). During 1989-90, we resampled four of the same field populations. Two of the populations were treated repeatedly with *Bt* between the first and second sampling (SO on Oahu and KH on the island of Hawaii). These heavily treated sites were compared with sites that received little or no *Bt* between the first and second sampling (WO on Oahu and LH on Hawaii). A watercress farm (NO on Oahu) that had been treated with Bt approximately 50-400 times was sampled in 1989 only (Fig. 1).

Larvae were reared on cabbage in the laboratory and F_1-F_3 offspring of field-collected DBM were tested for susceptibility to Dipel, a commercial formulation of *Bt*, using leaf residue bioassays. Larvae from two untreated laboratory colonies, LAB-P and LAB-L, were also tested

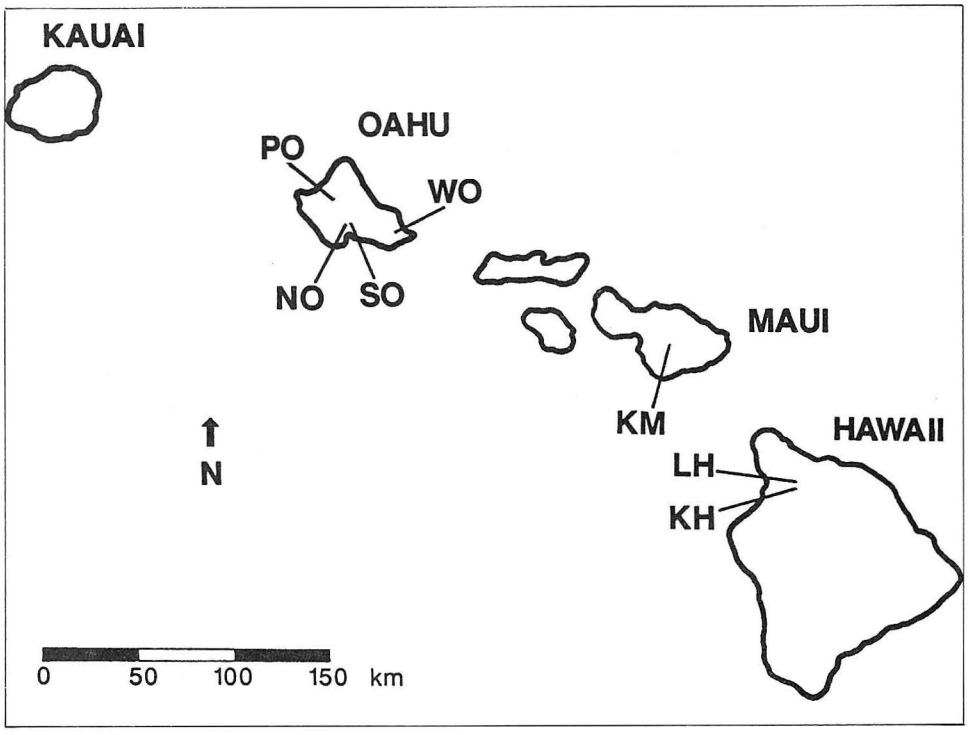

Fig. 1. Study sites (see Tabashnik et al. 1987, 1990 for detailed background information on study sites).

for susceptibility to *Bt* during 1986-87 and 1989-90. Mortality was recorded after 48 hours. All tests were replicated 4-16 times (see Tabashnik et al. 1990 for additional details).

Response to continuation and relaxation of selection: To assess the response of the resistant NO population to continuation and relaxation of selection in the laboratory, we first tested F_1 offspring of field-collected individuals using leaf residue bioassays (Tabashnik et al. 1991). The F_1-F_3 generations were reared without exposure to *Bt*. F_4 offspring were split into three selected subcolonies (NO-P, NO-Q, and NO-R) and one unselected subcolony (NO-U). For each generation of selection, approximately 200 larvae from each subcolony were fed leaf disks dipped in Dipel 2X at 25.6-512 mg active ingredient (AI) per liter which caused approximately 39-94% mortality. Larvae from NO-P, NO-Q, and NO-R were selected for five generations and tested by bioassay. Larvae from NO-P and NO-Q were selected for an additional four generations and then tested by bioassay again. The unselected subcolony (NO-U) was tested by bioassay at the 4th, 6th, 9th and 15th generations.

We also used bioassays to measure the response of the resistant SO population to relaxation of selection in the laboratory and field and to continuation of selection in the field. We collected DBM from SO on 18 May, 30 June, and 15 September 1989. We reared colonies initiated from the field samples of 30 June and 15 September without exposure to *Bt* for three and two generations, respectively, to determine their response to relaxation of selection in the laboratory. The SO field population was not treated with *Bt* between 30 June and 15 September. Response to relaxation of selection in the field was measured by comparing the suceptibility of larvae that were offsprings of individuals collected 30 June versus 15 September. The SO field population was treated with *Bt* five times between 18 May and 30 June. To measure the response to this additional selection, we compared the susceptibility of larvae obtained from individuals collected on 18 May versus 30 June.

Analysis. Median lethal concentrations (LC_{50}s) were estimated using probit analysis (PROC PROBIT, SAS 1985). Two LC_{50}s were considered significantly different if their 95% fiducial limits did not overlap (see Tabashnik et al. 1990 and 1991 for additional details).

Results

Field development of resistance. Our results showed that repeated treatments with *Bt* increased resistance to *Bt* in field populations of DBM (Fig. 2). The initial survey of field populations during 1986-87 showed that the SO population, which had been treated with *Bt* approximately 50-100 times from 1978 to 1982, was significantly more resistant to *Bt* than either of the susceptible laboratory strains (LAB-P and LAB-L). The LC_{50} of the SO population was about six times greater than the LC_{50}s of the LAB-P strain and the most susceptible field population, KM, a minimally treated population from Maui (Fig. 2). However, the LC_{50} of the SO population was not significantly greater than the LC_{50} of the two minimally treated populations on Oahu (WO and PO).

Between our initial survey and subsequent sampling during 1989-90, the SO population was treated with *Bt* 15 times and the KH population was treated repeatedly (number of treatments not known). Resistance to *Bt* in both of these populations increased significantly. The LC_{50} for SO doubled and the LC_{50} for KH quadrupled (Fig. 2). In contrast, during the same period, resistance to *Bt* did not increase significantly in two minimally treated field populations (WO and LH) nor in the two untreated laboratory populations (LAB-P and LAB-L). Results from 1989-90 showed that resistance in SO and KH, the two heavily treated populations, was significantly greater than resistance in WO and LH, the minimally treated populations.

The heavily treated NO population, which was sampled only in 1989, was highly resistant to *Bt* (Fig. 2). The LC_{50} for NO was 28 times greater than the average LC_{50} of the two laboratory colonies and 41 times greater than the LC_{50} of the most susceptible field population (KM).

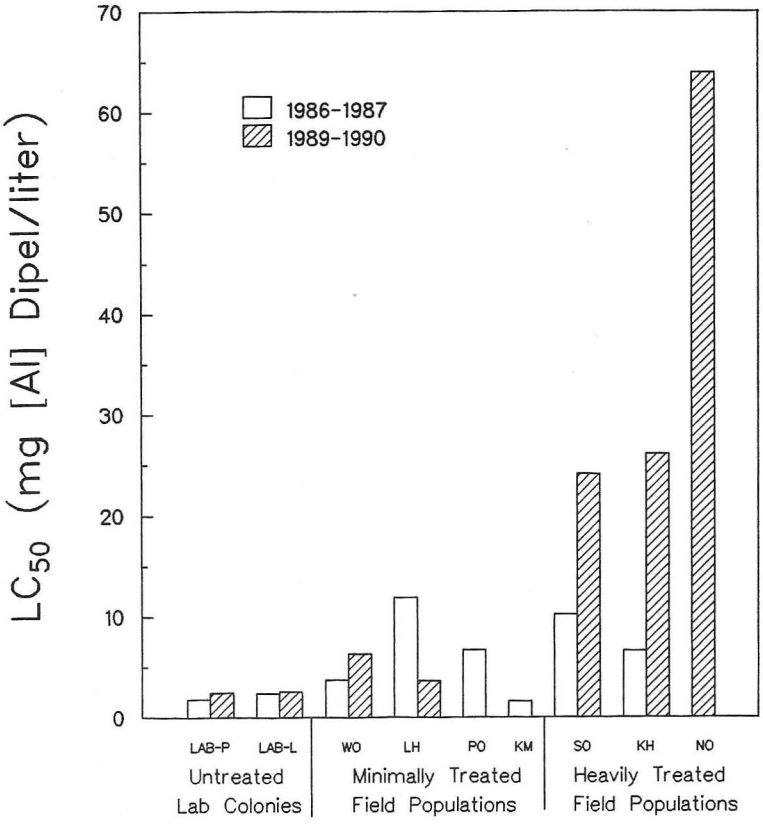

Fig. 2. Susceptibility of DBM larvae to *Bt* (Dipel). PO and KM were sampled only during 1986-87. NO was sampled only during 1989. KH received few *Bt* treatments before 1986, but was treated repeatedly between 1987 and 1990. The average slope of the concentration-mortality lines was 1.45 (range: 1.08 + 0.21 SE to 2.66 + 0.52 SE) (expanded from Tabashnik et al. 1990).

Response to continuation of selection. Laboratory selection with *Bt* rapidly increased resistance to *Bt* in each of the selected subcolonies (NO-P, NO-Q, and NO-R) (Fig. 3). Five generations of selection caused 5-7-fold increases in LC_{50}; nine generations of selection increased LC_{50}s by 15-30-fold. After nine generations of selection, LC_{50}s were 430-820 times greater than the LC_{50} of the susceptible LAB-P colony. Mortality at the field rate of *Bt* (25.6 mg (AI)/l) was less than 2% for the selected subcolonies compared with 90-100% for susceptible laboratory colonies.

In contrast to the rapid response to laboratory selection, five treatments of *Bt* applied to the SO field population caused no detectable increase in LC_{50}. The LC_{50} estimated from the 18 May field collection (before treatments) was 24 mg AI/l; the 30 June LC_{50} (after treatments) was 11 mg AI/l.

Response to relaxation of selection. Field-selected resistance to *Bt* declined slowly when treatment with *Bt* was stopped. In the laboratory, the LC_{50}s of the unselected subcolony initiated from NO (NO-U) were 64, 29, 18, 38, and 9.5 mg AI/l at the F_1, F_4, F_6, F_9, and F_{15} generations, respectively (Fig. 4). LC_{50}s for the F_6 and F_{15} were significantly lower than the initial LC_{50}. LC_{50}s for the F_4 and F_9 did not differ significantly from the original estimate.

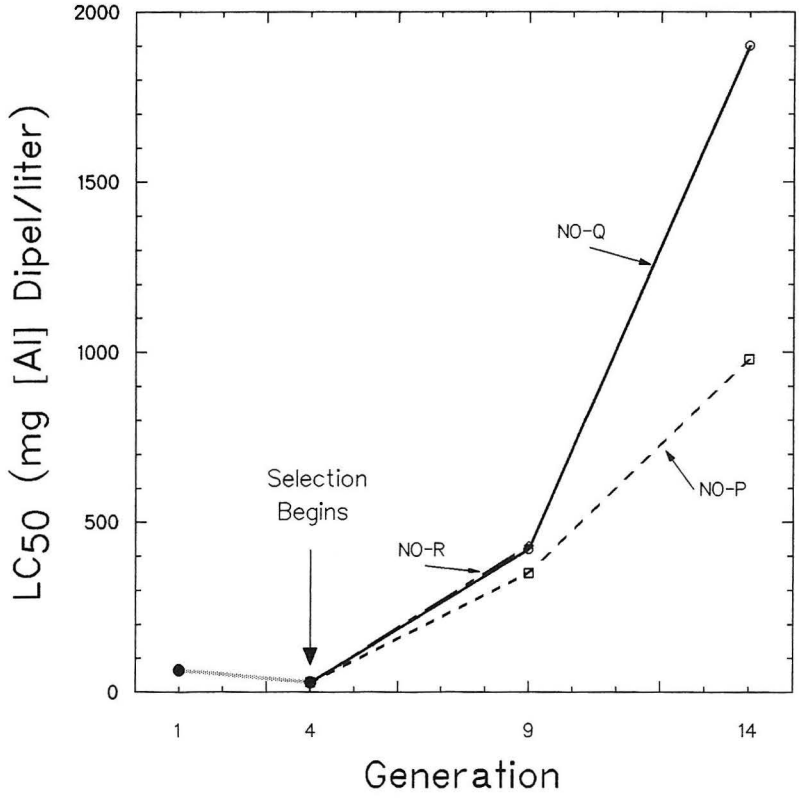

Fig. 3. Response of a field-selected DBM population to additional selection for resistance to *Bt* in the laboratory (adapted from Tabashnik et al. 1991).

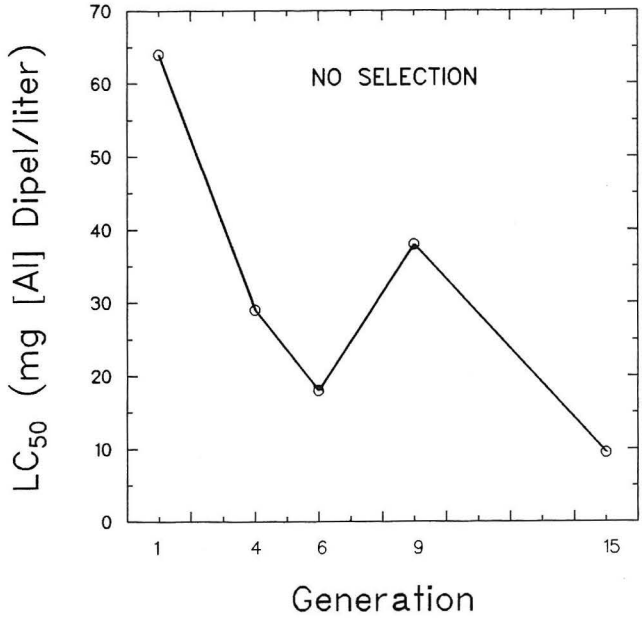

Fig. 4.
Response of a field-selected DBM population to relaxation of selection in the laboratory (adapted from Tabashnik et al. 1991).

For the colony initiated from the 18 May collection from SO, the LC_{50}s (all expressed in mg AI/l) were 48 for the F_1, 18 for the F_2, and 23 for the F_3. The LC_{50} for the 15 September collection from SO declined from 32 in the first generation to 17 in the second generation. None of the differences among laboratory-reared generations of SO colonies were significant.

Between 30 June and 15 September the SO field population was not treated with *Bt*. However, the LC_{50} for 15 September (24 mg AI/l) was not lower than the LC_{50} for 30 June (11 mg AI/l).

Discussion

Microbial pesticides, particularly *Bt*, are likely to become increasingly important as pest resistance and environmental concerns reduce the usefulness of conventional insecticides. Although laboratory selection has increased resistance to *Bt* in several species of insects (McGaughey 1985; McGaughey and Beeman 1988; Stone et al. 1989; Miller et al. 1990), the lack of documented cases of resistance to *Bt* in open field populations led to the assumption that such resistance was unlikely (Briese 1981; Wilcox et al. 1986; Wilding 1986; de Barjac 1987).

Our results show, however, that at least three field populations of DBM in Hawaii have evolved resistance to *Bt*. Each of these populations has been treated repeatedly with *Bt*. Indeed, the frequency of use of *Bt* to control these populations may have been comparable to excessive use of conventional pesticides. Thus, our results support Sun's prediction that if *Bt* is used like a conventional insecticide, then development of resistance to *Bt* can be expected (p. 398 in Talekar and Griggs 1986).

One field population (NO) with a history of frequent *Bt* treatments was highly resistant to *Bt* compared with other populations. We also documented substantial and statistically significant increases in resistance to *Bt* in two other field populations (SO and KH) in response to numerous treatments with *Bt* that were made between 1986-87 and 1989-90. Our data show that nearby field populations that were not treated repeatedly with *Bt* and two untreated laboratory colonies showed no comparable increase in resistance to *Bt* during the same period. In conjunction with growers' reports of reduced efficacy, our results provide strong evidence that field populations of DBM have developed resistance to *Bt* that is sufficiently high to thwart control.

Our finding of resistance to *Bt* in DBM populations occurring on two different islands (Oahu and Hawaii) and two different crops (watercress and cabbage) strongly suggests that resistance to *Bt* evolved independently at least twice in Hawaii. If so, we would anticipate that genes for resistance to *Bt* are also present in DBM populations outside of Hawaii. Thus, we would expect resistance to *Bt* to develop in other field populations of DBM that are treated repeatedly with *Bt*. Indeed, we are aware of suspected resistance to *Bt* in DBM from Asia and North America.

The rapid response to laboratory selection in our experiments showed that the resistant NO population contained genetic variation for resistance to *Bt*. We hypothesize that susceptible laboratory populations did not evolve resistance to *Bt* in previous selection experiments (Devriendt and Martouret 1976; Krieg and Langenbruch 1981) because they were relatively small and genetically homogeneous.

In our selection experiments, five generations of selection caused 5-7-fold increases in LC_{50}s. After nine generations of selection, LC_{50}s were 40-70 times greater than the recommended field rate of *Bt*. These results show that DBM has the potential to attain much higher levels of resistance than those previously found in the field.

In contrast to our laboratory results, we found that five treatments with *Bt* in the field did not cause a detectable increase in resistance to *Bt*. We hypothesize that the selection intensity imposed by five field treatments was weaker than that imposed by five generations of laboratory selection because more larvae escaped treatment in the field and concentrations of *Bt* used in the laboratory were higher than field rates. Other potential differences that might have affected the results include immigration and emigration in the field, and differences in initial genetic variation between populations.

In conclusion, resistance of DBM to *Bt* provides warnings about intensive use of microbial insecticides. First, DBM can evolve resistance to mixtures of *Bt* toxins. Field populations of

DBM developed resistance to Dipel, which contains a mixture of *Bt* toxins (Höfte and Whiteley 1989). We do not know if DBM resistance to Dipel is primarily or entirely due to resistance to one of the toxins in Dipel. Nonetheless, the ability of DBM to resist Dipel shows that mixtures of *Bt* toxins are not impervious to resistance development. Experiments are needed to determine if resistance to single toxins evolves more slowly than resistance to mixtures of toxins. Limited experimental evidence from conventional insecticides suggests that mixtures do not always retard resistance development (Tabashnik 1989; Immaraju et al. 1990).

Second, rotations or other management strategies that assume rapid restoration of susceptibility when *Bt* treatments are stopped may not be particularly useful. Our results show that field-selected resistance declined slowly and inconsistently when exposure to *Bt* was discontinued. Our data suggest that leaving several generations untreated may not always reduce resistance. Approximately 1 year of rearing the NO-U subcolony without exposure to insecticide caused a significant and substantial decline in its LC_{50} to *Bt*. Survival at the field rate of *Bt* decreased from 66 to 43%, but this was still much higher than the 0-10% survival of susceptible populations treated with the field rate. This decline in resistance would be too slow to markedly enhance the success of rotations.

Third, intensive use of *Bt* against resistant populations can cause rapid development of extremely high levels of resistance. The NO population, which had reached approximately 30-fold resistance in the field, quickly attained greater than 400-fold resistance when selected with high concentrations of *Bt* in the laboratory. These results suggest that attempts to overwhelm moderately resistant populations with high concentrations of *Bt* are likely to backfire. After resistance to *Bt* has increased to detectable levels, alleles for resistance to *Bt* are likely to be common enough to give the 'high dose' strategy little chance of suppressing resistance (Tabashnik and Croft 1982).

In summary, as in most cases, the best opportunity to manage resistance to *Bt* in DBM is to take action before resistance occurs. *Bt* should be used judiciously to conserve its efficacy against DBM. Management programs that emphasize biological and cultural controls can integrate *Bt* and other insecticides sparingly, thereby prolonging their usefulness.

We do not know if results from DBM can be extrapolated to provide insight into the potential for resistance to *Bt* in other pests. The most detailed knowledge of resistance to *Bt* currently available is derived from laboratory-selected strains of other insects, particularly *Plodia interpunctella* (Hübner), the Indian meal moth (McGaughey 1985; Johnson et al. 1990; van Rie et al. 1990). Because field- and laboratory-selected resistances can differ markedly, studies of field-selected resistance in DBM may be particularly valuable for understanding and managing resistance to *Bt*.

The remarkable success of insects in overcoming virtually every type of insecticide suggests that we would be wise to assume that the threat of resistance to *Bt* is imminent (Gould 1988a, 1988b; Raffa 1989). Underestimating our enemy could be costly; proceeding with caution may help to conserve the effectiveness of an extraordinarily specific and environmentally safe insecticide.

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Potential of Several Baculoviruses for the Control of Diamondback Moth and *Crocidolomia binotalis* on Cabbages

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Abstract

Restriction endonuclease (REN) analysis of viral DNA was used to characterize the diamondback moth, *Plutella xylostella* (L.), granulosis virus (PxGV), and to confirm cross-transmission of *Galleria mellonella* nuclear polyhedrosis virus (GmMNPV) to both diamondback moth and *Crocidolomia binotalis* (Zell.) and *Autographa californica* nuclear polyhedrosis virus (AcMNPV) to diamondback moth. Pathogenicity studies using neonate larvae showed PxGV to be most pathogenic to diamondback moth (LD_{50} 2-5 occlusion bodies (OB) per larva), followed by GmMNPV (LD_{50} 21-24 OB/larva) and AcMNPV (LD_{50} 26 OB/larva). GmMNPV was also pathogenic to *C. binotalis* (LD_{50} 3-10 OB/larva). LT_{50} data show that GmMNPV kills faster (LT_{50} 3.4 days) — followed by PxGV (LT_{50} 5.0 days) and AcMNPV (LT_{50} 5.1 days) — Glasshouse trials showed that PxGV sprayed at the rate of 8.9×10^{13} OB/ha gave good control of a synchronized population of diamondback moth. In trials in which molasses was included in PxGV sprays there was evidence of better population suppression and reduction in damage. The population suppression achieved with PxGV-molasses spray was better than a 10-fold higher PxGV dose, without molasses. The potential of each of the viruses for use in IPM programs is discussed.

Introduction

The control of pests of crucifers is heavily dependent on chemical pesticides. Continued dependence and usage over the years has resulted in problems of key pests such as diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), becoming resistant to nearly all the chemical pesticides being used. Use of increasing dosages as the chemicals used become less effective has given rise to problems of poisoning to farmers and residues on the crop produced. Increasing concern for the quality of the environment and the increasing costs of developing new compounds as older ones become less effective has encouraged research and investment in safer alternatives that can reduce pesticides usage to safe levels.

In Malaysia, parasitoids have been identified or introduced for the control of DBM (Lim and Ko 1975; Lim 1982). These parasitoids now play a central role in the current IPM program.

Fungal pathogens (*Zoophthora radicans*) have been recorded in the Cameron Highlands of Malaysia (Ooi 1981). However, no further work has been done to manipulate the fungus for controlling DBM.

Recently use of *Bacillus thuringiensis* Berliner (*Bt*) for DBM control on cabbages increased, mainly because nearly all the chemical pesticides being used for DBM control are no longer effective. Recent IPM trials carried out in the Cameron Highlands and the lowlands in Jalan Kebun in Malaysia have also shown that pesticide usage could be reduced drastically. In the

IPM packages tried, for most of the time there was only need to spray *Bt* (Syed et al. 1990), which did not have a direct adverse effect on parasitoids such as *Cotesia plutellae* and *Diadegma semiclausum*.

Several baculoviruses have been reported to be infective to DBM. Granulosis virus (GV) infecting DBM was first reported by Asayama and Osaki (1969). Since then several workers have reported that the GV shows promise as a control agent for DBM (Kao and Rose 1976; Wang and Rose 1978; and Nakahara et al. 1986). Viruses with a wider host range have also been reported to infect DBM. The *Autographa californica* MNPV (AcMNPV) is infective to DBM (Vail et al. 1972). Zeya (1968) reported on a nuclearpolyhedrosis virus that was isolated from DBM. The virus was also infective to several species of Lepidoptera. In a recent study, the virus was shown by using restriction endonuclease (REN) analysis to resemble *Galleria mellonella* NPV (GmMNPV) (Abdul Kadir and Payne 1989).

Although several viruses have been reported infective to DBM, the cross-transmission of the virus has not been confirmed using REN analysis of viral DNA. Some of these viruses have been reported to have potential for the control of DBM but data on the relative pathogenicity of these viruses to DBM is still lacking. This paper reports on the evaluation of several baculoviruses for the control of DBM and one of the viruses for control of *Crocidolomia binotalis* (Zell.). In Malaysia, *C. binotalis* occurs together with DBM on cabbages and can be important at certain times of the year (Sivapragasam 1981). It would therefore be an advantage to have a control measure that includes pests that occur together on the same crop.

Materials and methods

Virus characterization and cross-transmission. The viruses used in the present study were characterized using REN analysis of viral DNA. REN recognizes a specific nucleotide sequence in double-stranded DNA and cleaves them at or near the recognition sites (Nathan and Smith 1975). Thus one enzyme will consistently produce specific fragments from a specific DNA molecule. Virus cross-transmission to different hosts was also confirmed using the above technique.

Pathogenicity of viruses to DBM. Three viruses were used in the present study: PxGV, *Galleria mellonella* MNPV (GmMNPV) and *Autographa californica* MNPV (AcMNPV). Pathogenicity of the viruses to DBM was determined by using the droplet feeding method (Hughes and Wood 1981). To reduce variation in the test insects, neonate larvae were used. Larvae that hatched within a 2-hour period were further starved for another 3-4 hours before being offered virus suspensions at concentrations differing by 1/2 log. A red dye (Phenol red) was added to the virus suspension to identify larvae that had ingested the virus suspension. For each dose 25 test larvae were used. The volume of virus suspension taken up by test larvae needed to calculate the virus dose was determined using a fluorescent dye (Van Beek and Hughes 1986).

Data analysis was carried out using the maximum likelihood program (MLP program 308, NAG Ltd. Oxford). All data were probit transformed and estimates of median lethal concentration (LC_{50}) and 95% fiducial limits obtained. The volume ingested was used to calculate median lethal dosage (LD_{50}). Reproducibility of the assay procedure was examined using the test for parallel assays and the same program. Median lethal dosages (LD_{50}s), which allow more useful comparison of pathogenicity to be made, were used to compare pathogenicity of the viruses in the study.

Comparative susceptibility of DBM and *C. binotalis* to GmNPV. Susceptibility of both insect species to the GmNPV was compared using the assay procedure described above. Neonate larvae of both species were used as test insects. Median lethal dosage was used to compare the susceptibility of the two species to GmNPV.

Speed of kill of viruses. In addition to LD_{50}, speed of kill is another useful criterion for evaluating the potential of a virus. During the study, daily records of mortality due to viruses were kept for all bioassays. The median lethal time (LT_{50}) for all doses, based only on numbers of larvae responding to virus infection (Allaway and Payne 1984) was estimated from graphic plots of the time-mortality responses. Comparisons of LT_{50} for infections of the viruses in DBM were made.

Glasshouse trials using the PxGV to control DBM

The performance of PxGV for control of DBM on cabbages was investigated in two glasshouse trials. In the first trial, three PxGV dose rates were tested to determine dose rates that would give adequate control of DBM. The dose rates tested were 8.9×10^{11}, 8.9×10^{12} and 8.9×10^{13} GV occlusion bodies (OBs) per hectare. Cabbages (cv. Golden Acre) were infested with DBM by placing 10 eggs on each plant when they were 42 and 56 days old. Each test plot consisted of a 7×3 array of potted cabbages spaced 40 cm apart and replicated three times. In the second trial the effect of including molasses in PxGV sprays was investigated. In this trial cabbages were infested by placing 30 eggs on each plant when they were 40 days old. Three dose rates were tested in this trial: 9×10^{11}, 9×10^{12} and 9×10^{13} OB/ha (GV 11, GV 12 and GV 13 respectively) The second highest dose rate (9×10^{12} OB/ha) was used to test the effect of molasses. Each test plot in this trial consisted of an array of 2×8 cabbages spaced 40 cm apart. Each treatment was replicated three times.

Results

Characterization and cross-transmission

Characterization of inoculum and progeny viral DNA using REN analysis confirmed the cross-transmission of PxGV to DBM of the GmNPV to DBM and *C. binotalis* and of AcNPV to DBM. DNA profiles of the inoculum and progeny viruses are identical with respect to major bands.

Pathogenicity of viruses to DBM and *C. binotalis*

Pathogenicity of PxGV, GmNPV and AcNPV to DBM was expressed in terms of median lethal dosages (LD_{50}s). The results for the assay using the PxGV show LD_{50} values ranging from 1.9 to 5 occlusion bodies (OB) per larva (Table 1). For GmMNPV in neonate DBM the values ranged from 21.1 to 24 OB/larva, while the value was 26.2 OB/larva for AcMNPV (Table 2). The results based on LD_{50} values thus show that PxGV was most pathogenic to DBM. GmMNPV and AcMNPV do not differ significantly in their pathogenicity to DBM.

LD_{50} values for GmMNPV in neonate *C. binotalis* larvae ranges from 3.4 to 9.8 OB/larva. The mean value is about 6 OB/larva. *C. binotalis* is thus comparatively more susceptible to GmMNPV than DBM (LD_{50} values 21-24 OB /larva).

On the basis of the LT_{50}s, however, GmMNPV kills quicker than PxGV. For GmMNPV LT_{50} values were dose-dependent and ranges from 2.5 days for the highest dose to 4.3 days for the lowest dose (Table 3). For the PxGV, LT_{50} values ranged from 4.9 days for the highest dose and 5.4 for the lowest dose, and does not seem to be dose-dependent (Table 3). With the AcMNPV, LT_{50} value ranged from 3.8 days for the highest dose to 6.3 days for the lowest dose, and seems to be dose-dependent. The LT_{50} for GmMNPV infection in *C. binotalis* ranged from 3.8 days for the highest dose to 4.2 days for the lowest dose. Here again GmMNPV shows a faster speed of kill. The LT_{50} values were dose-dependent.

Results thus show that although based on LD_{50}, PxGV is most pathogenic to DBM, in terms of LT_{50}, GmMNPV kills faster.

Table 1. Mean median lethal dosages (LD_{50}s) for PxGV in neonate DBM larvae.

Assay no.	Group	Fiducial limits			Slope	SE of slope	Chi square	df
		LD_{50}	Lower	Upper				
1-8	1	1.9	1.6	2.3	1.37	0.069	79.530	50
4-12	2	3.0	2.5	3.6	1.46	0.086	69.369	54
10-14	3	5.0	3.9	6.5	1.44	0.131	36.150	29

Table 2. Mean median lethal dosages (LD_{50}s) of GmMNPV and AcMNPV in neonate DBM larvae.

Assay no.	LD_{50}	Fiducial limits		Slope	SE of slope	Chi square	df
		Lower	Upper				
			GmMNPV				
1-7	21.1	17.6	25.5	1.51	0.0876	57.586	46
2-8	24.0	19.7	29.1	1.51	0.0883	67.384	46
			AcMNPV				
1-3	26.2	18.1	37.2	2.11	0.276	33.976	15

Table 3. Mean median lethal times (LT_{50}s) for PxGV and GmNPV infections in neonate DBM larvae.

Log. conc.	LT_{50} (95% confidence interval) Days	
OB/ml	GmNPV	PxGV
5.0		5.4 (5.2-5.6)
5.5	4.3 (3.8-4.9)	5.0 (4.8-5.2)
6.0	3.7 (3.4-4.0)	4.9 (4.7-5.1)
6.5	3.6 (3.5-3.7)	5.1 (4.9-5.3)
7.0	3.4 (3.3-3.5)	4.8 (4.7-4.9)
7.5	3.1 (3.0-3.2)	4.9 (4.6-5.2)
8.0	2.5 (2.4-2.6)	

Efficacy for control of DBM

The first trial was carried out to determine effective dosage levels of the virus for control of DBM. The variates recorded were larval and pupal counts, number of virus-infected larvae and damage. Analysis of the data showed in many instances the effectiveness of the two higher virus concentrations tested in the control of DBM. In many instances also a significant linear response to virus dosages used in the trial was shown by the statistical analysis. In general, the data clearly showed the effect due to virus and the effectiveness of the two higher virus dosages in controlling a synchronized DBM population (Fig. 1 and 2).

In the second trial the effect of including molasses in virus sprays was tested. The effective lower dose rate (9×10^{12} OB/ha) shown in the first trial was selected to examine the effect of molasses on the efficacy of the virus for controlling DBM. Trial results revealed some interesting trends. The first sampling carried out, when virus infection can be observed, does not show significant differences between treatments, although the damage recorded was lower in plots sprayed with PxGV together with molasses (Fig. 3). Larval number was also lower (but not significantly lower) than other treatments. Numbers of virus-infected larvae recorded were highest in plots sprayed with PxGV together with molasses; higher even than plots sprayed with a 10-fold higher dosage of the PxGV (Fig. 3). The analysis of variance also showed a significant linear regression between virus infection and virus dosages used. The second sampling was carried out when pupation was observed. Damage recorded was again lowest in PxGV plots

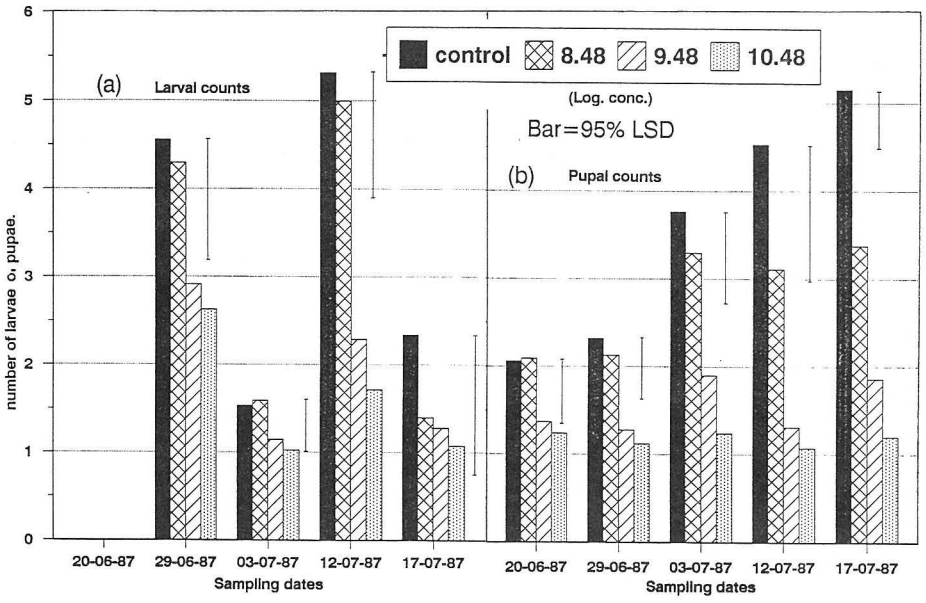

Fig. 1. Effect of PxGV dosages on DBM larval infestation and pupal survival on cabbages.

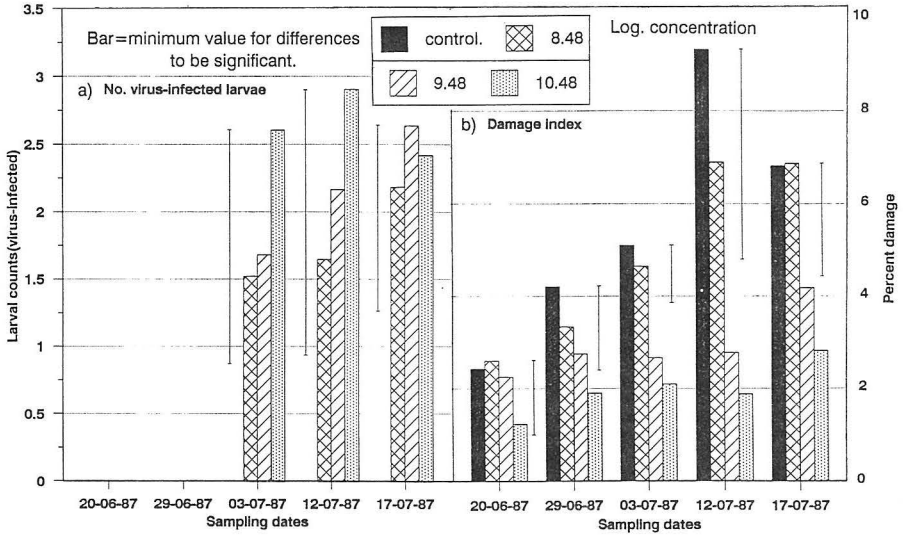

Fig. 2. Effect of PxGV dosages on a) virus infection and b) damage to cabbage leaves.

sprayed with molasses, with a significant linear effect of dose also shown (Fig. 3). Significantly lower damage in PxGV molasses plots compared to the control was recorded whereas differences were not significant between the other treatments and the control (Fig. 3). Virus infection had dropped significantly in PxGV-molasses plots, while for the other PxGV virus infection recorded was still high (Fig. 3). A significant linear effect of dose was also shown. The low virus infection was most likely attributable to larvae becoming infected, when very small, in PxGV-molasses

plots, and thus most of them had died by the time the second sample was taken. Further evidence to support this is the low damage recorded in PxGV-molasses plots (Fig. 3); larvae that become infected when very small (during the first instar) die small before they can inflict significant damage. The results suggest that molasses probably induced larvae to feed on droplets containing virus, and thus caused earlier and higher virus infection rates. Results of larval and pupal counts were consistent with damage and virus infection results. Larval counts were lowest in PxGV-molasses plots, whereas for the other PxGV-sprayed plots a significant linear response to virus dosages used was recorded. Similar trends were shown by the pupal counts.

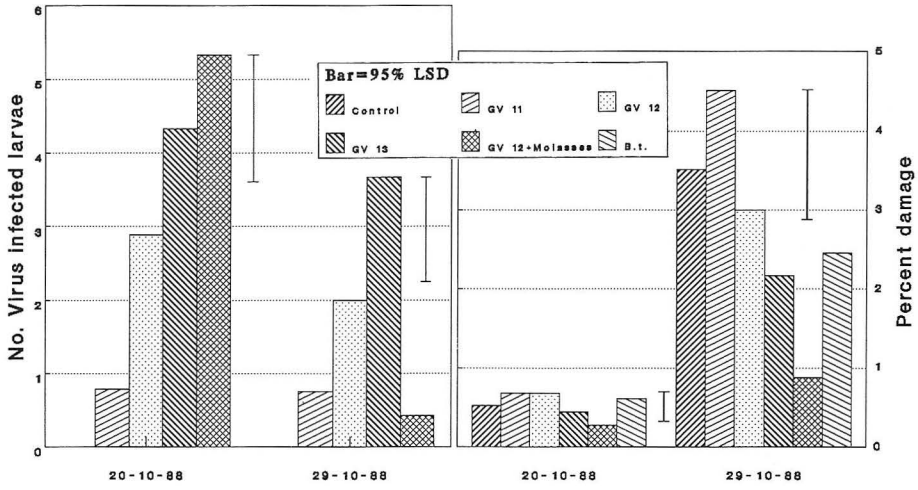

Fig. 3. Effect of PxGV dosages and molasses on virus infection and damage.

Discussion

Insect viruses have until recently been named after the host from which they were isolated. REN analysis of viral DNA has made it possible to accurately describe insect viruses. The technique is sensitive enough to enable different strains to be differentiated. It has also provided a means of unequivocally confirming true cross-transmission of a virus to alternate hosts. The GmMNPV was earlier described as NPV of DBM (Zeya 1968). Using REN the virus was shown to be an isolate of GmMNPV (Abdul Kadir and Payne 1989). The importance of accurate identification of insect viruses cannot be overemphasized. REN analysis of viral DNA is invaluable in the search for insect viruses or strains of the virus potentially useful for insect control. The technique is also useful in confirming true cross-transmission as opposed to the activation of a latent virus infection (McKinley et al. 1981). In host range studies the technique is invaluable; in the present study confirmation of cross transmission of GmMNPV to DBM and *C. binotalis* was shown. The in vivo bioassay is a fundamental tool in research on the use of microbial agents for pest control (Burges and Thompson 1971). The nature of virus infection is such that after ingestion by susceptible species, cessation of feeding does not occur. Feeding continues quite normally and damage continues to be inflicted. Younger larvae are also consistently more susceptible than older larvae (Payne 1982). As such, for the evaluation of insect viruses using the *in vivo* bioassay it is most appropriate to use neonate larvae. The information on dose mortality obtained would also be relevant to dosage requirements in the field.

Reproducibility of results is vital to prove their reliability. In the present study, results based on the parallel assay test showed that the bioassay technique is adequately reproducible. LT_{50}

values provide additional information. A virus that kills quickly will help reduce damage due to pests.

Several factors can influence the efficacy of viruses as control agents, including spray coverage, timing of spray, formulation and ultraviolet (UV) radiation. The most important is probably UV inactivation. The problem of virus inactivation by UV may be reduced in several ways. Addition of activated carbon or India ink in sprays has been shown to reduce inactivation by UV (Kao and Rose 1976). Results of the present study seem to indicate that molasses probably acted as a phagostimulant. Quicker uptake of virus perhaps could compensate for the fast inactivation of viruses by UV light. The value of molasses as a feeding stimulant has also been reported by Entwistle and Evans (1985).

From the present study it is recognized that the trial carried out in the glasshouse does not totally reflect conditions in the field in terms of exposure to UV, the presence of an unsynchronized population and also the complex of pest present. More work obviously needs to be carried out to test the virus in the field, especially the role viruses can play in IPM programs.

Most of the limitations that reduce the efficacy of viruses for pest control could probably be overcome with research. Other workers are also of the opinion that PxGV has potential for controlling DBM (Kao and Rose 1976; Wang and Rose 1976; and Nakahara et al. 1986). In reality the full potential of viruses as control agents has a better chance of being realized with the active participation of government agencies. Participation by the private sector will be determined by market size. Viruses with broader host range, such as AcMNPV, are attracting a lot of interest because of their better market potential. For GmMNPV, work will be continued to determine its host range for the complex of pests on brassicas such as DBM, *Hellula undalis* and *C. binotalis*, with the objective of determining its effectiveness for controlling a range of important pests that occur on brassicas. The most recent work has shown that GmMNPV is infective to *H. undalis*. Further laboratory and field work is needed to determine efficacy of the GmMNPV and AcMNPV for control of DBM, *H. undalis* and *C. binotalis* in the field in an IPM program. Host range and pathogenicity which affects its marketability will greatly influence the development for use in IPM programs.

The merits of a specific control agent such as a virus in IPM programs are well known. Viruses are compatible with beneficial organisms as well as chemical pesticides and thus ideal in IPM programs. It is therefore important for government to play a more active role in research involving microbial agents to ensure that the full potential of viruses in IPM programs is realized.

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22

Epizootics of *Pandora blunckii* and *Zoophthora radicans* (Entomophthoraceae: Zygomycotina) in Diamondback Moth Populations in the Philippines

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Abstract

Entomophthoralean fungi cause natural epizootics in populations of the diamondback moth, *Plutella xylostella* (L.), in the Philippines. Natural epizootics of *Pandora* (*Erynia*) *blunckii* (Lakon ex Zimm.) Humber and *Zoophthora radicans* (Bref.) Batko were observed. The fungi can infect over 95% of the larvae and over 70% of the pupae on cabbage (*Brassica oleracea*); on petchai (*B. campestris*) the DBM density and the fungus infection levels were lower, i.e. only 20% of the larvae and 30% of the pupae were infected. There was no correlation between climatic data, i.e. relative humidity, rainfall, and temperature and the occurrence of the epizootics. The epizootics occurred after the DBM populations had reached their highest numbers. Insect density is probably an important factor for the initiation of the fungal epizootics in Philippine DBM populations.

Introduction

Insect fungi

Entomopathogenic fungi are specific natural enemies of insects and mites. The fungi are predominantly found in the Hyphomycetes (Deuteromycotina, with teleomorphs in the Ascomycotina) and the Entomophthoraceae (Zygomycotina). They infect their hosts by penetration through the cuticle and growth of hyphae in the host cavity. The fungi occur worldwide on crop pests such as those of soybeans (Ignoffo 1981) and citrus (McCoy 1981), on insects of medical importance such as mosquito larvae (Jaronski 1990) and on insects in forests, particularly in tropical rain forests (Evans 1982; Rombach and Roberts 1989).

Insect fungi can cause epizootics (epidemics) that decimate insect populations. They often control pest populations when: (a) the environmental conditions, in particular relative humidity and temperature, favor fungal development, and (b) the insect host is present in sufficient numbers to sustain such an epizootic. Lately increased efforts are being made to use fungi for the control of various insect and mite pests such as the citrus rust mite in Florida (McCoy 1981), vine weevils (*Otiorhynchus sulcatus* L.) on ornamentals in Europe (Zimmermann 1984) and the brown planthopper (*Nilaparvata lugens* (Stahl)) on rice in Asia (Rombach et al. 1987).

Novel mass production methods, such as the marcescent process (Soper and Ward 1981) and improved growth media (Trinci et al. 1990), were developed to produce large amounts of relatively inexpensive fungus inoculum to be used in field trials.

Fungi on DBM

Entomophthoralean fungi were collected from diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), around the world (Wilding 1986). Velasco (1983) found *Zoophthora radicans* (Bref.) Batko (Entomophthoraceae: Zygomycotina) on larvae of DBM in the Philippines.

Recently we collected *Pandora blunckii* (Lakon ex Zimm.) Humber (Entomophthoraceae: Zygomycotina) (Riethmacher and Kranz 1991), *Z. radicans* and a hitherto undescribed *Conidiobolus* species (Entomophthoraceae: Zygomycotina) from DBM larvae and pupae in the Philippines (Riethmacher et al. 1990). The development of epizootics of entomophthoralean fungi in DBM populations as well as fungus mass production and field trials were investigated. Results of the epizootiology studies are partly reported here.

Materials and Methods

Survey methods

Field sites. Surveys were carried out on (a) cabbage plants, *Brassica oleracea* L. (cv. 'Scorpio') as well as on (b) a local nonheading cabbage ('Petchai'; *B. campestris* L.). The survey fields (about 150 each) were adjacent and on the grounds of the Benguet State University (BSU), La Trinidad, Baguio City. The fields received normal maintenance (i.e. weeding, low level fertilization, adequate irrigation), but were not treated with insecticides or fungicides. The surveys were made during the dry season (November 1989-January 1990).

Survey techniques. Sixty randomly selected cabbage plants and 240 petchai plants were marked at the start of the surveys. On these plants the living and infected DBM larvae and pupae were counted weekly (but not collected). A total of 10 surveys on cabbage and five surveys on petchai were made. The numbers of living and infected larvae and pupae were added separately. The petchai field survey data were recalculated to represent numbers per 60 plants to be able to compare insect populations on petchai with the populations on cabbage.

Collection and incubation

Field site. The field was situated adjacent to the fields used for the surveys. It was transplanted to cabbage at the same time as the survey fields and received the same maintenance treatment. The collections were carried out during the same period as the surveys.

Collection. The collection fields were visited weekly. Each week 15 plants were selected randomly and 15 DBM larvae (second and third instar) were collected from these plants and transferred to separate plastic containers.

Incubation. In the laboratory the larvae were transferred to small 'Bellaplast' containers (9 cm diam) on a cabbage leaf disc. The leaf discs were washed before the incubation to prevent possible infection of the larvae by fungi present on the leaves. A wet cotton plug was added to the container to ensure maximum relative humidity. The temperature remained at about 20°C for the incubation period.

Evaluation. The larvae were examined daily for the development of fungus diseases. Larvae which developed a fungus disease were removed and the fungus was identified. A percentage infection of the 225 larvae collected per sampling date was calculated.

Weather data

The weather data were provided by the PAGASA weather station (Benguet State University); the station is situated directly at the border of the experimental fields. Only data on temperature, rainfall, and relative humidity are presented here.

Results

Survey results

Cabbage. At the first counting date (11 November 1989) only a very few DBM larvae (about 100 larvae/60 plants) were present (Fig. 1). The population increased to about 4000 larvae/60 plants after 7 weeks, but close to harvesting (10 weeks after transplanting) the larval population decreased dramatically. The pupal population peaked at 7 weeks after transplanting at about 250 pupae/60 plants (Fig. 2).

Fungus infections first occurred at 6 weeks in the larval and at 7 weeks in the pupal population. Infection levels increased up to 95% in the larva population (Fig. 1) and up to 70% in the population of pupae (Fig. 2).

Petchai. DBM infestations on the petchai were considerably lower compared with the cabbage (Fig. 3 and 4). In the petchai field larvae and pupae were found after 3 weeks. The population increased to approximately 220 larvae/60 plants (Fig. 3) and 12 pupae/60 plants (Fig. 4). Seven weeks after transplanting, fungus infections had increased to about 20% in the larval population and 30% in the pupal population.

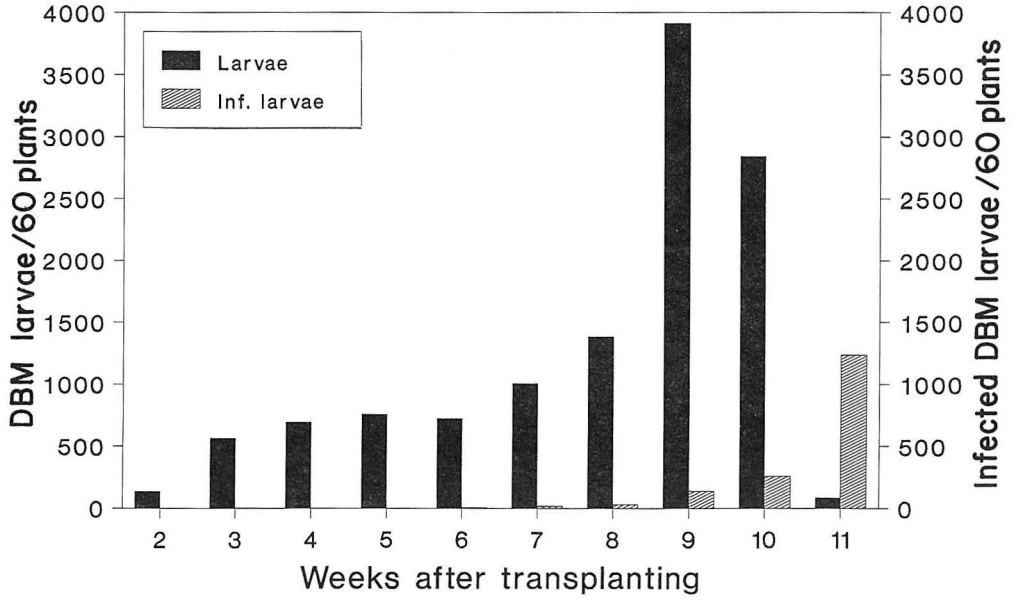

Fig. 1. Numbers of healthy and fungus-infected DBM larvae on cabbage at different observation dates.

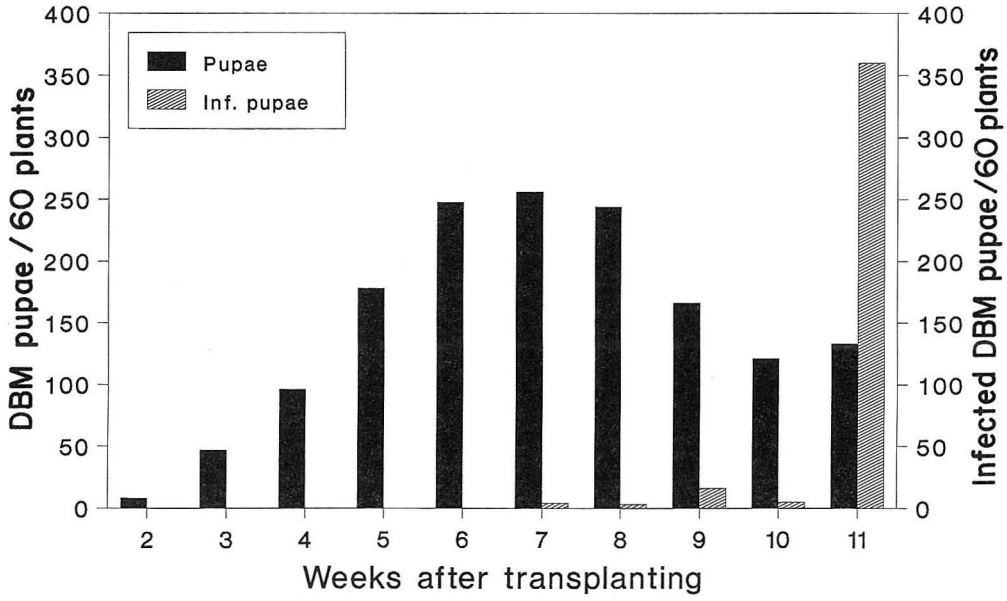

Fig. 2. Numbers of healthy and fungus-infected DBM pupae on cabbage at different observation dates.

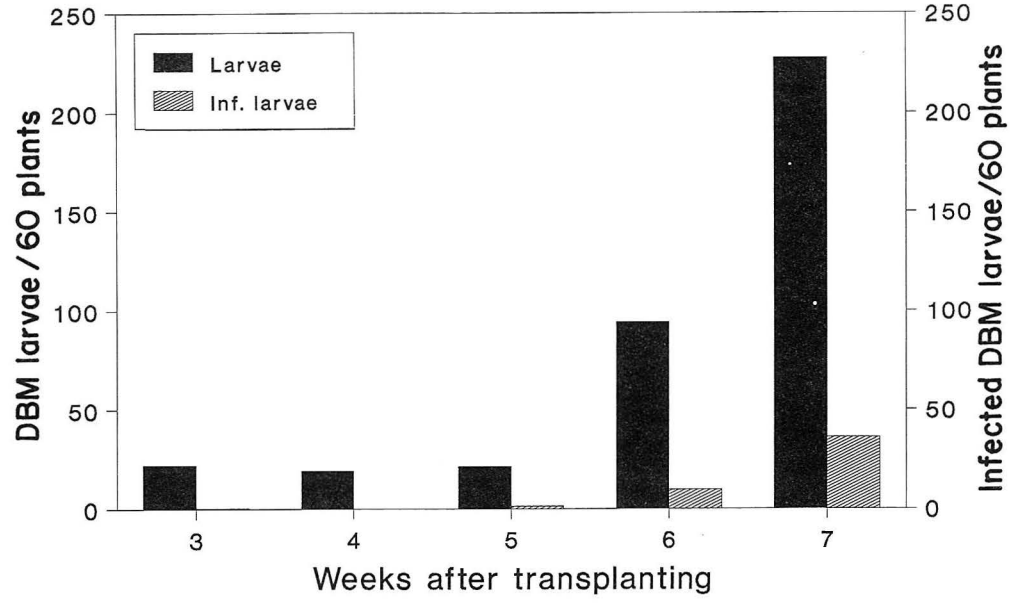

Fig. 3. Numbers of healthy and fungus-infected DBM larvae on petchai at different observation dates.

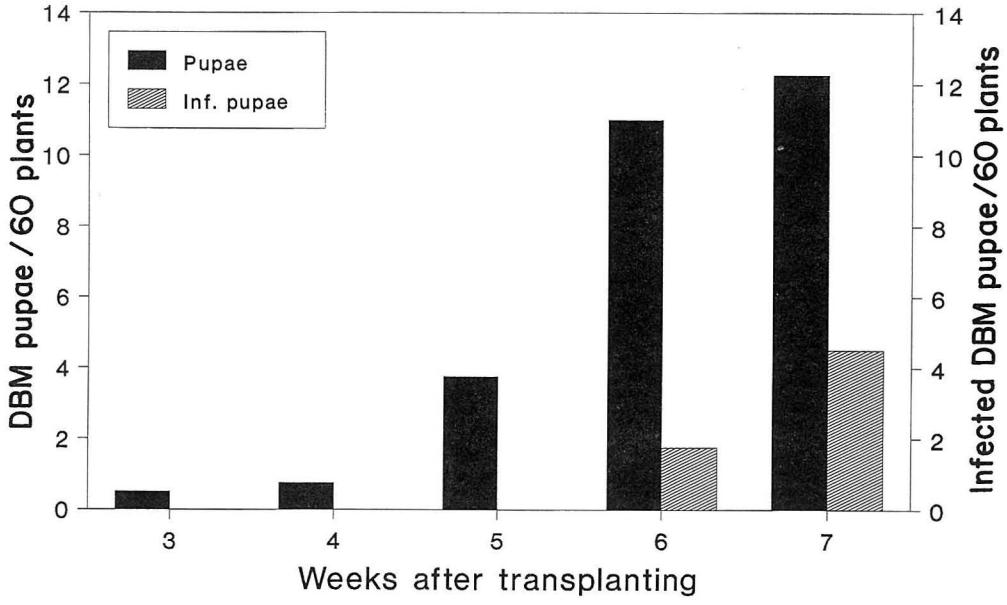

Fig. 4. Numbers of healthy and fungus-infected DBM pupae on petchai at different observation dates.

Weather data

The average maximum temperature was about 23 °C, and the average minimum temperature was 12 °C (Fig. 5). No apparent trends in temperatures were present. Only one strong rain-shower occurred during the studies.

The average relative humidity was high, and never below 65%; average nightly relative humidity mostly exceeded 85%. No apparent trends in relative humidity were present during the studies.

Collection and incubation

Pandora blunckii and *Z. radicans* were the only fungi identified from the field-collected and incubated larvae (Table 1). Infections with *P. blunckii* increased from 1 to 22% during the sampling period. *Z. radicans* occurred in the last collection, but only on 4% of the larvae.

Table 1. Infestation of field-collected DBM larvae by *Pandora blunckii* and *Zoophthora radicans* after incubation in the laboratory.

Sampling date	Larvae (%) infected with	
	P. blunckii	*Z. radicans*
13 November 1989	1	0
27 November 1989	5	0
11 December 1989	12	0
8 Janurary 1990	12	0
19 January 1990	22	4

Conclusions

Entomophthoralean fungi clearly are important natural enemies of DBM on cabbage and petchai in the Philippines. *P. blunckii* is the dominant fungus in these surveys and collection

fields; *Z. radicans* was collected on 19 January 1990 (the last sampling date) and only infected 4% of the larvae. In all, the fungi infected up to 95% of the larvae on cabbage, and up to 20% of the larvae on petchai in the surveys.

The lower infection rates on the petchai plants might be caused by the lower insect density on the petchai (Fig. 3 and 4). The lower insect infestation probably reflects a preference for cabbage of the DBM adult, since the fields were adjacent and adults could easily move between them. Also, petchai plants are considerably smaller compared to cabbage, which affects population growth rates.

The fungi only occurred at a fairly late stage in the plant development, i.e. shortly before harvest and before the DBM populations decreased. However, from this survey we cannot determine whether the decline of the populations was due to the fungus infections or to other factors.

It might well be that high DBM populations have to be present to initiate the fungus epizootics. This is unfortunate because the damage often occurs before these epizootics. It should be investigated whether artificial dissemination of fungus material at lower insect densities can cause the epizootics to occur at an earlier stage of the DBM population development. Kelsey (1965) achieved infections of DBM larvae in New Zealand by field application of cadavers infected with *Z. radicans*.

Field trials to test the efficacy of mass-produced Entomophthoraceae to control DBM populations on cabbage are now being conducted in the Philippines. Mass-produced material of these fungi might well become a new tool for DBM management, but only if epizootics can be induced at lower DBM infestation levels.

Acknowledgments

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BIOLOGICAL CONTROL

Role of Parasitoid Complex in Limiting the Population of Diamondback Moth in Moldavia, Romania

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Abstract

The complex of parasitoid and hyperparasitoid species which is associated with diamondback moth (*Plutella xylostella* (L.)), a destructive pest of cabbage in Romania, is presented. Over 25 species of Ichneumonidae and Braconidae are identified, which act as primary and secondary parasitoids. For each species, abundance, constancy, dominance, the biocenotic affinity and the contribution of each species in limiting the population of diamondback moth is analyzed. The dynamics of these species over time, from one crop to another and from one area to another are discussed. Also discussed are those species which play a role in biological control of this pest, and those which could be used in biocontrol.

Introduction

Diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) is one of the most serious pests of cabbage in Romania. In Moldavia this species completes two or three generations in one crop season (Peiu et al. 1971, 1973). The greatest damage occurs when the infestation takes place in young plants. In older plants the larvae develop mainly on the outer leaves. Such damage is of little economic significance. Larvae from the first and second generations cause the greatest damage in Moldavia.

Our research was aimed at clarifying the complex of parasitoids that limited the population of DBM.

Materials and Methods

The research was conducted in three stages: 1967-72, 1979-82 and 1990. In the first stage, we emphasized identification of the parasitoid species and their interrelationship. In the second and third stages, the importance of each species within the biocenotic complex and the contribution of each species in limiting populations of DBM was considered.

Based on the material collected from over 40 localities in Moldavia during the study period, we found that DBM was controlled by a complex of parasitoid species that reduced the populations of this pest by 80-90% or even more.

We collected 16,961 mature larvae and pupae of DBM. They were reared in the laboratory for adult parasitoid emergence, identification and synecological studies.

Results and Discussion

Of the 16,981 larval specimens collected from 1967 to 1972 (Table 1), 1514 larvae and pupae (8.9%) died due to diseases and insecticides. A total of 4777 pupae emerged into DBM

adults (28.2%). The balance of the collection, 10,670 specimens, emerged into parasite adults. They belonged to 28 species, as follows.

Table 1. The synecological analysis of the parasitoid species in the populations of DBM.

Species	Abundance	Dominance	Constancy	Index of ecological significance
Diadegma fenestralis	3,182	18.76 D5	88 C4	16.50 W5
Diadegma armillata	1,773	10.45 D5	79 C4	8.25 W4
Diadegma chrysosticta	1,749	10.31 D5	76 C4	7.83 W4
Diadegma vestigialis	754	4.44 D3	52 C3	2.30 W3
Diadromus subtilicornis	623	3.67 D3	45 C2	1.65 W3
Diadegma cerophaga	560	3.30 D3	62 C3	2.04 W3
Diadromus collaris	364	2.14 D3	42 C2	0.89 W2
Diadromus ustulatus	347	2.04 D3	38 C2	0.77 W2
Diadegma tibialis	251	1.47 D2	44 C2	0.64 W2
Diadegma trochanterata	227	1.33 D2	43 C2	0.57 W2
Apanteles fuliginosus	215	1.26 D2	39 C2	0.49 W2
Diadegma gracilis	81	0.47 D1	20 C1	0.09 W1
Diadegma gibbula	54	0.31 D1	15 C1	0.04 W1
Diadegma holopyga	41	0.24 D1	26 C2	0.06 W1
Diadegma interrupta	39	0.22 D1	16 C1	0.03 W1
Itoplectis alternans	36	0.21 D1	27 C2	0.05 W1
Phaeogenes ischiomelinus	36	0.21 D1	16 C1	0.03 W1
Dicaelotus parvulus	32	0.18 D1	19 C1	0.03 W1
Itoplectis viduata	25	0.14 D1	18 C1	0.02 W1
Itoplectis tunetanus	24	0.14 D1	11 C1	0.01 W1
Diadegma monospila	22	0.12 D1	14 C1	0.01 W1
Hyposoter ebeninus	8	0.04 D1	7 C1	0.002 W1
Apanteles ruficrus	8	0.04 D1	8 C1	0.003 W1
Apanteles rubecula	4	0.02 D1	4 C1	0.0008 W1
Nepiera moldavica	3	0.01 D1	1 C1	0.0001 W1

Primary parasitoids

A. Family Ichneumonidae: 1. *Itoplectis viduata* Grav., 2. *I. tunetanus* Schm., 3. *I. alternans* Grav., 4. *Nepiera moldavica* Const. and Must., 5. *Diadegma armillata* Grav., 6. *D. cerophaga* Grav., 7. *D. chrysosticta* Gmel, 8. *D. fenestralis* Holmgr., 9. *D. gibbula* Brsch., 10. *D. gracilis* Grav., 11. *D. holopyga* Thoms., 12. *D. interrupta* Holmgr., 13. *D. monospila* Thoms., 14. *D. tibialis* Grav. 15. *D. trochanterata* Thoms., 16. *D. vestigialis* Rtzbg., 17. *Hyposoter ebeninus* Grav., 18. *Dicaelotus parvulus* Grav., 19. *Diadromus subtilicornis* Grav., 20. *D. ustulatus* Holmgr., 21. *Thyraeella collaris* Grev., 22. *Phaeogenes ischiomelinus* Grav.

B. Family Braconidae: 1. *Apanateles fuliginosus* Wesm., 2. *A. rubecula* Marsh., 3. *A. ruficrus* (Hal.).

C. Family Pteromalidae: *Dibrachys cavus* (Walk.). D. Family Eulophidae: 1. *Tetrastichus* sp., 2. *Geniocerus* sp.

Secondary parasitoids

A. Family Ichneumonidae: *Mesochorus vittator* Zett., of *Diadegma armillata* Grav.; *Lysibia varitarsus* of *Apanteles fuliginosus* (Wesm.).

B. Family Eulophidae: *Pleurotropis* sp. of *Diadegma armillata* and *Apanteles fuliginosus*; C. Family Pteromalidae: *Eupteromalus* sp. of *Diadegma armillata*.

The action of the secondary parasitoids limits the efficiency of the primary parasitoids in controlling DBM populations. However, their presence is negligible and has no significant economic impact.

The interrelations between the species of this biocenotic complex are shown in Fig. 1.

Studies of parasitoid species identified in this complex (Table 1) indicate that there is considerable variation in these parasite species. Their high numbers and the high rate of parasitism puts *D. fenestralis* (18.8%) in the first place, followed by *D. armillata*, *D. chrysosticta*, *D. vestigialis*, *Diadromus subtilicornis*. Other species play a minor role in reducing host populations.

The relation between the parasitoid species varies from sample to sample in the same locality, from time to time during the year, from year to year and from area to area.

The large number of species that seem to parasitize DBM raises a legitimate question: Is each species closely associated with this host, or have some species reached this parasite complex more or less accidentally? To answer this question we carried out a synecological analysis of the populations of parasitoids within the biocenotic complex. Table 1 lists abundance, dominance, constancy and the index of ecological significance for each species.

The species are listed according to their abundance. The highest value is assigned to *D. fenestralis* with 3182 individuals, followed by *D. armillata* with 1773 individuals and *D. chrysosticta* with 1749 individuals. The lowest values were assigned to *A. rubecula* and *Nepiera moldavica* with only three individuals each.

To judge the importance of the presence of parasitoid species within this complex, we carefully analyzed their constancy as a structural indicator because this indicates the contribution of a species participating in the realization of the structure of the biocenosis.

From the ecological parameters we can deduce that species *D. fenestralis*, *D. armillata* and *D. chrysosticta* act as euconstant parasitoids, whereas *D. vestigialis* and *D. cerophaga* act as constant parasitoids. This means that all these species can be found in all the cabbage fields from Moldavia, wherever DBM attacks cabbage. Eight species act as accessory parasitoids and the other species can be considered as accidentally present in this complex.

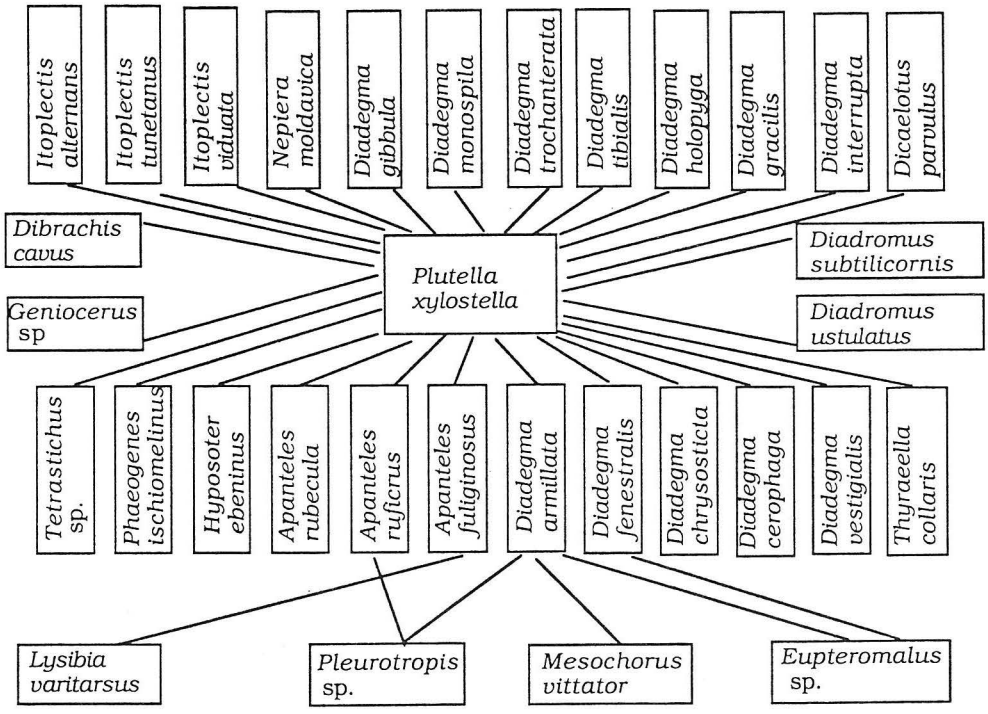

Fig. 1. The complex of parasitoids which limits the population of DBM in Moldavia, Romania.

Dominancy shows the number relationship of the individuals of a given species in contrast to the number of individuals of the other species they associate with. It indicates the relative abundance. The dominance parameter illustrates the participating degree of each species to the realization of biomass production in biocenosis. The species *D. fenestralis*, *D. armillata* and *D. chrysosticta* are eudominant; *D. vestigialis* is dominant; *Diadromus subtilicornis*, *D. ustulatus*, *Diadegma cerophaga* and *Diadromus collaris* are subdominant, followed by three recedent species and all the others subrecedent.

The index of ecological significance (W) represents the relationship between the structural and productive indicators. This shows more eloquently the position of each species in the complex. In this respect, the highest value is that of *D. fenestralis*, followed by *D. armillata* and *D. chrysosticta* with W_4 and by *D. vestigialis*, *D. cerophaga* and *Diadromus subtilicornis* with W_3, after which there follows five species with W_2, the others having reduced values.

Synecological analysis of the data obtained from our research indicates that only a few species make a major contribution in limiting populations of DBM to over 80%. This fact has important practical significance.

In order to convince ourselves that this complex of parasitoid species limits the populations of DBM, we continued our research (Mustata 1979, 1987; Mustata and Tudor 1973; Mustata and Lacatusu 1973).

Our initial survey from 1967 to 1979 identified all the parasite species that interact with the populations of this pest (Table 2). These parasitoid species considerably reduce the populations of DBM. The degree of parasitism varied between 13.9% in Ciurea, on 21 July 1971 and 95.6% in Ungureni on 21 August 1972. In most of the samples the percentage of parasitism is quite high, averaging 60.9%.

The value of the ecological parameters resulting from the analysis of the data obtained from 1979 to 1982 is shown in Table 3.

The data from the last study period (1990) are summarized in Table 4. Here, too, the same parasitoid species manifest themselves as being euconstant or constant, eudominant or dominant, and have a high index of ecological significance although there is reversal in the order and value of the ecological parameters.

We also analyzed the index of biocenotic affinity, the value which confirms the affinity between the main species derived from results obtained in the first stage.

The role of the main parasitoid species in limiting the populations of DBM in Adjudu Vechi and their dynamics in time and space are shown in Fig. 2. The number of DBM adults that emerged was very low. During 1969-71, the DBM emergence was close to 30%, whereas in 1972, 1979, 1980 and 1981 it was 4%. In 1972, it reached 69.5% and in 1990 it declined to 17.9%.

Diadegma armillata, *D. fenestralis*, and *D. chrysosticta* seem to be the major parasitoids limiting the populations of DBM. Other species of primary parasitoids with a clean competition between them also limit the populations of DBM.

Our results on the dynamics of the main parasite species at various locations in Moldavia during 1969 are summarized in Fig. 3, and for 1990 in Fig. 4. The localities shown in Fig. 4 are listed according to their geographical position from the south (Homocea) to the north (Mestecanis) of Moldavia. There is no relationship between the geographic location and populations of DBM or its major parasites.

On the basis of our research we could deduce that populations of DBM in Moldavia are limited by an important complex of natural enemies, their efficiency being very high (about 63%). Some of the parasitoid species are constant or euconstant in populations of DBM. The more important species are *D. fenestralis*, *D. armillata*, *D. chrysosticta* , *D. vestigialis*, *D. cerophaga*, and *Diadromus subtilicornis*. The combined parasitism of these major species and certain minor ones play an important part in DBM control in Moldavia. However, we often found that chemical insecticides were used despite levels of parasitism of almost 90%. In July 1972 in Adjudu Vechi and July 1970 in Scheia, farmers used chemicals to combat DBM although the samples taken only 8-10 days earlier showed only 4-5.5% DBM adult emergence. The balance,

Table 2. The synecological analysis of the parasitoid species in the period 1967-72.

Abundance	Dominance	Constancy	Index of ecological significance	Species	Biocenotic affinity																									
					Diadegma fenestralis	*Diadegma armillata*	*Diadegma vestigialis*	*Diadegma chrysosticta*	*Diadegma cerophaga*	*Diadromus subtilicornis*	*Diadromus ustulatus*	*Diadegma trochanterata*	*Diadegma tibialis*	*Apanteles fuliginosus*	*Diadegma gracilis*	*Diadromus collaris*	*Diadegma holopyga*	*Diadegma interrupta*	*Itoplectis alternans*	*Diadegma gibbula*	*Itoplectis tunetanus*	*Itoplectis viduata*	*Phaeogenes ischiomelinus*	*Diadegma monospila*	*Dicaelotus parvulus*	*Hyposoter ebeninus*	*Apanteles ruficrus*	*Nepiera moldavica*	*Apanteles rubecula*		
1,470	15.71 D_5	90 C_4	14.13 W_5	*D. fenestralis*	66	73	71	66	39	40	40	36	23	16	27	22	11	30	16	12	16	12	14	22	10	10	1	4	1	
1.183	12.64 D_5	77 C_4	9.73 W_4	*D. armillata*		51	60	62	48	36	56	34	21	18	28	26	14	31	18	18	21	14	16	20	6	14	2	2	2	
637	6.80 D_4	56 C_3	3.80 W_3	*D. vestigialis*			62	48	32	33	41	24	18	17	22	17	15	20	17	11	25	12	15	27	16	9	5	3	3	
582	6.21 D_4	77 C_4	4.78 W_3	*D. chrysosticta*				56	42	46	38	29	21	24	25	15	9	23	15	13	19	14	14	23	9	14	0	4	4	
366	3.91 D_3	69 C_3	2.69 W_3	*D. cerophaga*					42	31	51	34	23	20	21	29	18	38	20	27	17	13	15	13	7	12	2	2	5	
345	3.68 D_3	43 C_2	1.58 W_3	*D. subtilicornis*						36	41	29	31	17	28	17	11	17	10	32	28	26	7	16	12	16	0	8	6	
283	3.02 D_3	41 C_2	1.23 W_3	*D. ustulatus*							35	34	12	39	29	22	11	18	14	18	10	16	11	30	12	12	4	9	7	
182	1.94 D_2	52 C_3	1.00 W_3	*D. trochanterata*								40	30	18	17	18	20	36	18	18	19	17	16	14	10	9	3	0	8	
143	1.52 D_2	39 C_2	0.59 W_2	*D. tibialis*									5	25	24	28	12	26	24	24	15	22	7	22	18	8	4	4	9	
89	0.95 D_1	28 C_2	0.26 W_2	*A. fuliginosus*										13	16	4	0	28	19	34	20	10	4	17	5	23	0	6	10	
68	0.72 D_1	18 C_1	0.12 W_2	*D. gracilis*											4	16	13	17	8	5	18	23	21	22	7	6	0	0	11	
30	0.32 D_1	24 C_1	0.07 W_1	*D. collaris*												20	5	15	9	21	4	5	0	25	20	11	7	7	12	
29	0.30 D_1	22 C_1	0.06 W_1	*D. holopyga*													38	12	16	16	11	13	5	29	14	6	0	0	13	
27	0.28 D_1	13 C_1	0.03 W_1	*D. interrupta*														28	22	16	11	13	16	11	14	6	0	0	14	
26	0.27 D_1	30 C_2	0.08 W_1	*I. alternans*															14	6	6	6	16	16	11	0	14	0	15	
26	0.27 D_1	18 C_1	0.04 W_1	*D. gibbula*																12	35	13	4	26	7	15	5	0	16	
23	0.24 D_1	16 C_1	0.04 W_1	*I. tunetanus*																	11	11	14	6	15	15	0	0	17	
16	0.17 D_1	11 C_1	0.02 W_1	*I. viduata*																		11	6	6	10	7	10	1	18	
16	0.17 D_1	13 C_1	0.01 W_1	*P. ischiomelinus*																			25	23	5	15	0	0	19	
14	0.14 D_1	11 C_1	0.01 W_1	*D. monospila*																				8	5	10	0	0	20	
14	0.14 D_1	24 C_1	0.03 W_1	*D. parvulus*																					13	0	0	8	21	
6	0.06 D_1	9 C_1	0.003 W_1	*H. ebeninus*																						0	0	25	22	
6	0.06 D_1	11 C_1	0.006 W_1	*A. fuficrus*																							0	14	23	
3	0.03 D_1	1 C_1	0.0003 W_1	*N. moldavica*																								0	24	
2	0.02 D_1	2 C_1	0.0004 W_1	*A. rubecula*																									25	

Table 3. The synecological analysis of the parasitoid species in the period 1979-82.

Species	Abundance	Dominance	Constancy	Index of ecological significance
Diadegma chrysosticta	792	17.11 D5	88 C4	15.05 W5
Diadegma fenestralis	723	15.62 D5	92 C4	14.37 W5
Diadegma armillata	267	2.77 D4	96 C4	5.53 W4
Diadromus collaris	192	4.15 D3	80 C4	3.32 W3
Diadromus cerophaga	149	3.22 D3	68 C3	2.15 W3
Diadegma subtilicornis	98	2.12 D3	52 C3	1.10 W3
Diadegma tibialis	87	1.88 D2	60 C3	1.12 W3
Apanteles fuliginosus	74	1.60 D2	68 C3	1.08 W3
Diadegma vestigialis	67	1.45 D2	48 C2	0.69 W2
Diadromus ustulatus	52	1.52 D2	40 C2	0.44 W2
Diadegma trochanterata	36	0.78 D2	36 C2	0.28 W2
Diadegma gibbula	24	0.52 D1	8 C1	0.04 W1
Phaeogenes ischiomelinus	20	0.43 D1	40 C2	0.17 W2
Dicaelotus parvulus	18	0.39 D1	28 C2	0.10 W2
Diadegma interrupta	10	0.22 D1	28 C2	0.06 W1
Diadegma gracilis	9	0.19 D1	16 C1	0.03 W1
Itoplectis alternans	8	0.70 D1	28 C2	0.19 W2
Itoplectis viduata	7	0.15 D1	28 C2	0.04 W1
Diadegma holopyga	7	0.15 D1	24 C1	0.03 W1
Diadegma monospila	6	0.13 D1	20 C1	0.02 W1
Hyposoter ebeninus	2	0.04 D1	8 C1	0.003 W1
Itoplectis tunetanus	1	0.02 D1	4 C1	0.0008 W1
Apanteles ruficrus	1	0.02 D1	4 C1	0.0008 W1
Apanteles rubecula	1	0.02 D1	4 C1	0.0008 W1

Table 4. The synecological analysis of the parasitoid species in the year 1990.

Species	Abundance	Dominance	Constancy	Index of ecological significance
Diadegma fenestralis	979	32.89 D5	100 C4	32.89 W5
Diadegma chrysosticta	375	12.60 D5	71 C3	8.94 W4
Diadegma armillata	323	10.85 D5	78 C4	8.96 W4
Diadromus subtilicornis	180	6.04 D4	57 C3	3.44 W3
Diadromus collaris	142	4.77 D3	57 C3	2.71 W3
Diadegma vestigialis	50	1.68 D2	57 C3	0.95 W2
Diadegma cerophaga	45	1.57 D2	42 C2	0.65 W2
Apanteles fuliginosus	43	1.44 D2	42 C2	0.60 W2
Diadegma tibialis	21	0.70 D1	50 C2	0.35 W2
Diadromus ustulatus	12	0.40 D1	35 C2	0.14 W2
Diadegma trochanterata	9	0.30 D1	35 C2	0.10 W2
Diadegma holopyga	5	0.16 D1	28 C2	0.04 W1
Diadegma gibbula	4	0.12 D1	21 C1	0.02 W1
Diadegma gracilis	4	0.12 D1	21 C1	0.02 W1
Itoplectis viduata	2	0.06 D1	14 C1	0.008 W1
Itoplectis alternans	2	0.06 D1	14 C1	0.008 W1
Diadegma monospila	2	0.06 D1	14 C1	0.008 W1
Diadegma interrupta	2	0.06 D1	14 C1	0.008 W1
Apanteles ruficrus	1	0.03 D1	7 C1	0.002 W1
Apanteles rubecula	1	0.03 D1	7 C1	0.002 W1

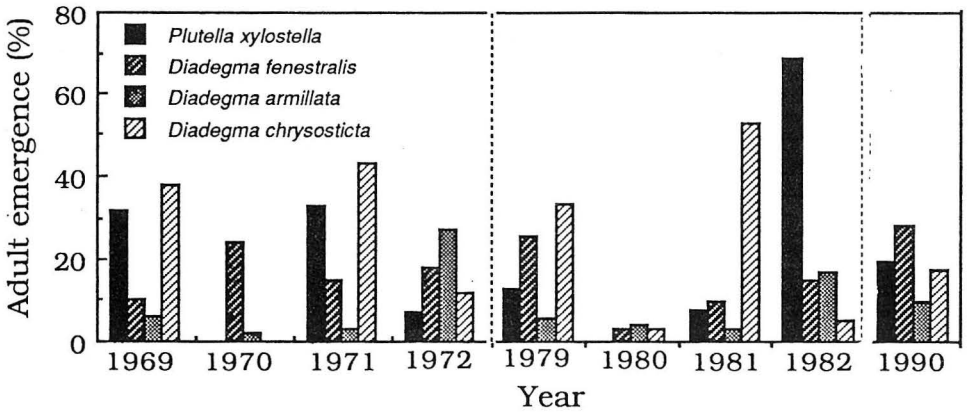

Fig. 2. Population dynamics of DBM and its major parasitoids during 1969-72, 1979-82 and 1990 at Adjudu Vechi.

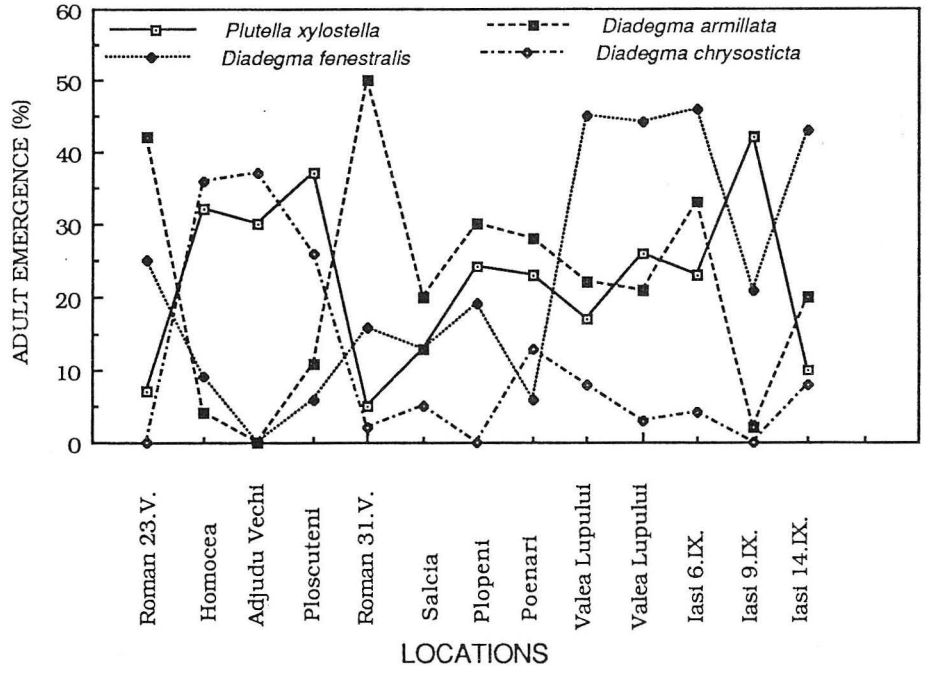

Fig. 3. Population dynamics of DBM and its major parsaitoids at various locations during 1969.

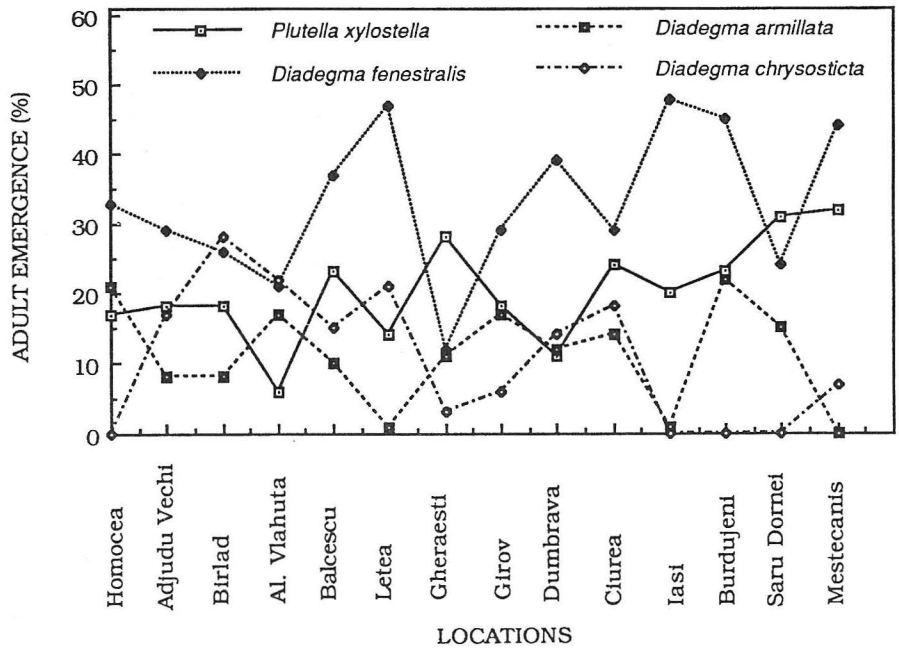

Fig. 4. Population dynamics of DBM and its major parasitoids at various locations during 1990.

over 95%, were parasitized. In these circumstances, treatment with insecticides is not justified, and adversely affects beneficial fauna with unpredictable consequences.

To combat pests we must know precisely the activity of the parasitoid and predatory species which limit pest populations. It is important that our interventions in natural ecosystems be done on the basis of thorough biocenotic data. Our intervention must be made in such a way that it does not affect the beneficial fauna. Otherwise we can provoke unpredictable disturbances in the biological equilibrium.

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Biological Control of Diamondback Moth in the Pacific

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Abstract

The diamondback moth *Plutella xylostella* (L.) is the major pest of brassicas in the oceanic Pacific, where it is often associated with the cabbage cluster caterpillar *Crocidolomia pavonana* (F.) and, less frequently, with the cabbage webworms *Hellula undalis* (F.) and *H. rogatalis* Hulst. *Cotesia plutellae* Kurdjumov (and, at most, no more than one other major parasitoid in any one country) is present in the Pacific, but only in 4 of the 20 or so countries. *Diadegma semiclausum* Hellen, possibly the most effective parasitoid of diamondback moth elsewhere, is not yet established in the Pacific, although there are current introductions to Cook Islands and Fiji. There are good reasons for believing that wider dispersal of those parasitoids present and the establishment of additional species would confer important benefits. The biological control of all important members of the brassica pest complex deserves early attention in order to minimize the need to apply insecticides, with their generally adverse effects on natural enemies.

Introduction

By far the most important insect pest of cabbages and other brassicas in the oceanic Pacific is the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae). Although extremely widespread, it is still apparently absent from Kiribati, Tokelau, Tuvalu, Wallis and Futuna (Waterhouse 1985), the Federated States of Micronesia (Esguerra et al. 1990) and also from the Marshall and Caroline Islands.

Cabbage is the most important brassica grown in the Pacific. The cabbage cluster caterpillar *Crocidolomia pavonana* (F) (= *C. binotalis*) is also a widespread pest of brassicas, but is less important than DBM. Somewhat less important still are the two cabbage webworm species *Hellula rogatalis* Hulst (in Micronesia) and *H. undalis* (F.) (throughout most of the Pacific).

Most of the other insects associated with cabbage only attain pest status locally and occasionally except, perhaps, for highly polyphagous species such as the corn earworm *Helicoverpa armigera* (Hübner), cutworms of the genus *Agrotis* and, less frequently, the armyworm *Spodoptera litura* (F) (Waterhouse and Norris 1987). The significance to the biological control of DBM of other insects attacking brassicas lies of course in the fact that, if insecticides need to be employed against them, these insecticides, unless selective, are likely to have serious adverse effects on the natural enemies of DBM.

The Present Position

The distribution and importance ratings assigned by country experts to the more damaging pests of cabbage in the Pacific are shown in Table 1. The damage ratings are largely subjective and different authorities from the same country do not always agree. Nevertheless, the ratings

Table 1. Distribution and importance of brassica pests in the oceanic Pacific.

	Major pests				Occasional Pests	
	DBM	*Crocidolomia pavonana*	*Hellula*		*Helicoverpa armigera*	*Agrotis* sp.
			undalis	*rogatalis*		
Cook Islands	+ + + *	+ +	+ +		?	+ +
Fiji	+ + + *	+ +	+		+ +	+ +
French Polynesia	+ + *	+	P		+	+
Federated States of Micronesia			+		+ +	
Guam	+ *	+ + +	+ + +		+ + + *	
Hawaii	+ + +		+ +			+
Kiribati					+	P
Marshall Islands						
New Caledonia	+ + + *	+	+		+ + + *	+
Niue	+ + + *	+ + + *	+ + + *		P	
Northern Mariana Islands	+ + + *	+ +	+ + +		+ + +	
Palau	+ + + *	+ + + *		+ + + *	+ + + *	
Papua New Guinea	+ + + *	+ + + *			+ + +	+ +
American Samoa	+ + + *	+ +	P			
Western Samoa	+ + + *	+			+	
Solomon Islands	+ +	+ + +	+ + *		+ +	
Tokelau			P			
Tonga	+ + + *	+ + + *	P		+ + + *	+
Tuvalu					P	P
Vanuatu	+ +	+ +			+ +	+
Wallis and Futuna						

*One of the country's top 10 insect pests; + + + widespread, causing important damage every year; + + less widespread, but of great importance; + important locally; P present, but unimportant.

do provide the best available guide to the relative importance of the pests. The same conventions have been used as in 'Biological Control: Pacific Prospects' (Waterhouse and Norris 1987). New data have been provided from a 1990 survey carried out by B.M. Thistleton, Biological Control Officer, South Pacific Commission, and from my recent correspondence with Pacific officials.

A workshop on biological control in the Pacific, held in Tonga in 1985 and attended by representatives of 15 Pacific nations, unanimously placed DBM at the top of the priority list of pests causing serious problems and deserving of an attempt at biological control. No improvement in pest status has taken place over the past 5 years, as shown in Table 1, that would alter this priority. Indeed, with the exception of a modest, continuing program in Fiji and a very recent introduction to Cook Islands (see later), there have been no active biological control projects involving DBM in this period. This situation may well change, however, since, at an SPC/GTZ Biological Control Planning Meeting in Fiji in July 1989, DBM was tentatively selected as the priority target for a major new initiative in the Pacific to commence during August 1990. It is thus timely to review what is known about the natural enemies of DBM in the Pacific, the history of past introductions of agents to the region and also to consider what the prospects are for successful biological control.

Since both Australia and New Zealand have benefited significantly from biological control of DBM, brief overviews are first presented to assist in setting the scene for consideration of the position in the oceanic Pacific.

Australia

DBM became a troublesome pest in Australia soon after it was first reported in 1893. It has since been the target of a number of attempts at biological control and three major parasitoids have been established (Table 2). It is also attacked by a number of native parasitoids (Waterhouse and Norris 1987). As a result, there has been a marked reduction in damage in many areas and particularly in the Australian Capital Territory and South Australia, where DBM now seldom needs to be treated with pesticides. Elsewhere, and particularly in Queensland, damage to crops for human consumption may require treatment but, for forage crops, less frequently than before. However, even if DBM were to become no problem, much the same regime of insecticide treatments would still be required against other brassica pests, including *Pieris rapae* (L.), *C. pavonana*, *Helicoverpa armigera*, *Hellula hydralis* Guene and *Brevicoryne brassicae* (L.). It is probable that the current insecticide regime exercises a significant adverse effect on the abundance of DBM parasitoids. Therefore, any renewed attempts at biological control should also involve the introduction of agents for all important members of the pest complex.

Even at the time of greatest pest pressure it is generally only necessary to spray brassicas at 7-14-day intervals and sometimes, as in winter, even less frequently. There has been an increase in *Bacillus thuringiensis* Berliner usage, but no growers appear to be relying on it entirely.

Table 2. Major parasitoids of DBM estabished in Oceania, Australia and New Zealand.

	Cotesia plutellae	*Diadegma insulare*	*Diadegma semiclausum*	*Diadromus collaris*	*Oomyzus sokolowskii*
Cook Islands	+			+	
Fiji	+				+
Hawaii	+	+		+	
Papua New Guinea	+				
Australia	+		+	+	
New Zealand			+	+	

New Zealand

In spite of the fact that they are attacked by hyperparasitoids, the introduction and establishment of *Diadegma semiclausum* (= *D. eucerophaga*) and *Diadromus collaris* (Table 2) has provided adequate control of DBM in North Island brassica crops, but insecticide applications are still sometimes necessary in the South Island. The fungus *Zoophthora radicans* Brefeld (= *Entomophthora sphaerosperma*) also exerts important control when weather conditions in autumn are suitable and moth populations high. In neither island do parasitoids keep damage at an acceptably low level in brassicas (especially cabbages) for human consumption. The major insecticide groups are still effective and the commercial use of *B. thuringiensis* preparations is rare. The establishment of additional parasitoids is under consideration (Thomas and Ferguson 1989).

Cook Islands

The two lepidopterous pests of cabbage in Cook Islands are DBM and *Crocidolomia pavonana*, the latter being of lesser importance. *Diadegma semiclausum*, *Diadromus collaris* and *Trichogramma* sp. were introduced in 1974-75 from New Zealand but, in 1978, only *D.*

collaris could be recovered (Table 3) (Walker and Deitz 1979). *Diadegma semiclausum* was again introduced from New Zealand in 1990, but there is no information yet on its establishment (G. Hill pers. comm. 1990).

Although it has not been intentionally introduced to Cook Islands *C. plutellae* was found on Raratonga (and possibly Mauke) in 1988 and, together with *D. collaris*, was responsible for moderate to low levels of parasitization. In addition, a male of *Apanteles* sp. (*ultor* group) was found in association with cabbages on Raratonga (G. Hill pers. comm. 1988). An *Apanteles* sp. of the same group has been reported from *C. pavonana* in Pakistan (CIBC 1981, 1982).

Resistance to organophosphorus insecticides emerged in the late 1970s and to synthetic pyrethroids in the mid 1980s. Trials with insect growth regulators have given good results, but these materials are not yet available. *Bacillus thuringiensis* has given variable results and more effective formulations are required (1990 Report of the Totokoitu Research Station).

Fiji

DBM continues to be a major pest in Fiji, where it is resistant to all insecticides that have been used against it for any length of time. Current recommendations are to spray with *B. thuringiensis* and chlorfluazuron (S.N. Lal pers. comm. 1990).

Diadromus collaris was introduced from New Zealand in 1943 (Lever 1943), but there is no record of its establishment (Table 2) (Rao 1971). Similarly, releases in 1971 of *C. plutellae*, *Macromalon orientale* and *D. collaris* from India failed to achieve establishment (Kamath 1979). Releases in 1984 of *Cotesia plutellae* and *Oomyzus* (= *Tetrastichus*) *sokolowskii* (which acts both as a parasitoid and a hyperparasitoid) have led to both species becoming established. Mass rearing and release of *C. plutellae* continued from 1984 until 1989, but was then discontinued to make way for the importation of *D. semiclausum* from Taiwan.

Although there are no survey data to establish percentage parasitization in the field due to *C. plutellae* and *O. sokolowskii*, both are well established and have spread to other cabbage-growing areas from their release sites (S.N. Lal pers. comm. 1990). In an unsprayed cabbage plot at Koronivia Research Station, 60-70% of DBM larvae were parasitized by *C. plutellae* (Kumar et al. 1987).

Guam

Between 1971 and 1975 four parasitoids (Table 3) were introduced either from India or Hawaii (*C. plutellae*, *Diadegma insulare*, *Diadromus collaris* and *O. sokolowskii*), but none of these established (R. Muniappan pers. comm. 1990). The braconid *Chelonus blackburni* Cameron, known also from Hawaii, has been reported attacking DBM (Nafus and Schreiner 1989). Since 1987, even where pesticides have not been used on experimental plots, DBM has occurred as a pest only very sporadically and only during dry seasons (R. Muniappan pers. comm. 1990).

It appears that *H. rogatalis* does not occur on Guam nor the Northern Marianas, but *S. litura* is a serious problem on cabbage on both, and also on Yap (R. Muniappan pers. comm. 1990).

Hawaii

DBM has been present in Hawaii at least since 1892 (Mitchell 1985) and continues to be an important pest of cabbage and other brassicas, except where densities remain below 0.5 larva/plant for the entire growing season. Many producers routinely apply insecticides to control this and other cabbage pests, but few compounds are now effective. Natural enemies provide valuable control at times when relatively high levels of damage can be tolerated, such as prior to formation of the cabbage head (Johnson et al. 1988; Tabashnik 1986).

Table 3. Introductions for the biological control of DBM in the oceanic Pacific.

	Liberated	From	Result	Reference
Cook Islands				
Diadegma semiclausum (Hellen)	1974-75	New Zealand	–	Walker and Deitz 1979
	1990	New Zealand	?	G.Hill pers. comm. 1990
Diadromus collaris (Gravenhorst)	1974-75	New Zealand	+	Walker and Deitz 1979
Trichogramma spp.	1974-75	New Zealand	–	Walker and Deitz 1990
Fiji				
Diadegma semiclausum	1945	New Zealand	–	Lever 1944, Oatman 1978 a
	1990	Taiwan	?	S.N. Lal pers. comm. 1990
Diadromus collaris	1943	New Zealand	–	Lever 1946, Oatman 1978 Rao 1971
	1971	India	–	Kamath 1979
Cotesia plutellae	1971	India	–	Kamath 1979
Kurdjumov	1972	Trinidad	–	Cock 1985
	1984	Trinidad	+	Anon 1986, Cock 1985
Macromalon orientale Kerrich	1971	India	–	Kamath 1979
Oomyzus sokolowskii	1984	Trinidad	+	Anon. 1986
Papua New Guinea				
Diadegma semiclausum	?	Australia	–	Simmonds 1971
Cotesia plutellae	1983	Hawaii	+	Lim 1986a
	1983	Malaysia	+	Lim 1986a
Tonga				
Diadromus collaris	1943	New Zealand via Fiji	–	Oatman 1978, O'Connor 1949, Simmonds 1971
Guam				
Diadromus collaris	1975	India	–	R. Muniappan, unpub.
Diadegma insulare	1975	Hawaii	–	R. Muniappan, unpub.
Cotesia plutellae	1971-72	India	–	R. Muniappan, unpub.
Oomyzus sokolowskii	1973	India	–	R. Muniappan, unpub.
Hawaii				
Cotesia plutellae	1972	Taiwan	–	Davis 1972, 1974, Lai et al. 1982 Funasaki et al. 1988b
	1971-73	Trinidad	–	Mitchell 1985
	1980	Trinidad	+	Lai et al.1982, Mitchell 1985
	1983	Taiwan	+	Lai and Funasaki 1986
Brachymeria boranensis Masi	1953	Kenya	–	Weber 1954
Diadegma insulare (Cresson)	1953	Kenya	+	Johnson et al. 1988. Mitchell 1985
Diadegma semiclausum	1985	Pakistan	–	Funasaki et al. 1988a
Diadromus collaris	1972	Trinidad	–	Mitchell 1985
	1983	Pakistan	–	Lai and Funasaki 1986
Oomyzus sokolowskii	1953	Kenya	–	Mitchell 1985
Trichogramma chilonis Ishii	1984	Taiwan	–	Lai and Funasaki 1986

The first parasitoid reported to attack DBM was *D. insulare* (identified at the time as *Limnerium polynesiale*) (Swezey 1915). However this important parasitoid of DBM in North America, which had appeared unaided, did not maintain pest density at subeconomic levels in Hawaii. Nor did the two other parasitoids more recently reported (Johnson et al. 1988), namely *Chelonus blackburni* and *Pristomerus hawaiiensis* Ashmead, which also appeared unaided and were recorded from a number of lepidopterous larvae as early as 1915 (Swezey 1915). They also attack *H. undalis* in Hawaii (Awai 1958). A race of *Diadegma insulare* from Kenya was released in 1953, but this did not achieve control. Two other species, *Brachymeria boranensis* and *Tetrastichus* nr *sokolowskii* (Table 3) were also introduced at the same time, but failed to become established (Mitchell 1985).

Cotesia (= *Apanteles*) *plutellae* was introduced on several occasions from 1972 onwards, but establishment was not achieved until 1982 (Lai and Funasaki 1985). This species is now widespread. Unsuccessful attempts have been made to establish *D. collaris* from Trinidad and Pakistan, *Diadegma semiclausum* from Pakistan and *Trichogramma chilonis* from Taiwan (Table 3).

Field studies on Maui in 1984 and 1985 (where DBM was the major cabbage pest) showed that combined parasitization of larvae by *C. plutellae* and *D. insulare* could rise as high as 59%. In spring, *D. insulare* was the major parasitoid, with *C. plutellae* playing a very minor role, whereas in summer and autumn their relative importance was reversed. In 1986 and 1987, sampling on Oahu, Maui and Hawaii showed that total parasitization could range up to 73.2%, although *D. insulare* was found only at sites higher than 780 m (Johnson et al. 1988).

It is interesting that DBM was effectively controlled on a watercress farm in Oahu through the use of an intermittent overhead water sprinkler, combined with the establishment of *C. plutellae*. Other pests were controlled by timely applications of diazinon (Nakahara et al. 1986).

Kiribati

Although Chinese cabbage, cabbage and other Brassicaceae have been widely grown in recent years, DBM does not occur in Kiribati. However, living larvae have been intercepted by strict quarantine inspections on imported cabbage (G.S. Sandhu pers. comm. 1990).

New Caledonia

There have apparently been no attempts at biological control. However, a pupal parasitoid *Brachymeria* sp. (Chalcididae) and a fungus (*Zoophthora* ? *radicans*) have been recorded attacking DBM which is the major pest of Brassicaceae (Delobel 1978).

Papua New Guinea

In unsprayed cabbage plots at Wau, which sustained an unacceptable level of damage, Gagne (1979) reported a *Brachymeria* sp. and another chalcidid wasp as pupal parasitoids and the vespid wasp *Ropalidia bambusae* Richards and larvae of syrphid flies as predators. Pupae were attacked by an unidentified fungus. Six wasp parasitoids [*Eriborus* sp., *Diadegma* sp. and? *Itoplectis* sp. (Ichneumonidae), *Agathis* sp. (Braconidae), *Brachymeria phya* (Walker) (Chalcididae) and an unidentified wasp] and a tachinid fly (*Compsilura* sp.) were reported at Mt Hagen (B.M. Thistleton pers. comm. 1985).

The only record of introductions is of *C. plutellae* from Hawaii and Malaysia prior to 1983. In 1984 rates of parasitization were still low and the level of biological control inadequate (Lim 1986a), a conclusion reiterated in 1987 (Thistleton 1987).

Tokelau

DBM does not occur in Tokelau where there are strict quarantine measures to exclude it and other pests (L. Naseri pers. comm. 1990).

Tonga

Sampling of DBM larvae on Tongatapu did not reveal any parasitoids. *Crocidolomia* larvae would still require treatment even if DBM were effectively controlled, but *Hellula* is only a minor pest. *Bacillus thuringiensis* is recommended, but is too expensive for farmers to use. Following the development of resistance to permethrin, fenvalerate and methamidophos are recommended, together with interplanting with onions, carrots, garlic, etc. (O. Fakalata pers. comm. 1990).

Tuvalu

Although Chinese cabbage is grown widely DBM does not occur (S. Seluka pers. comm. 1990).

Vanuatu

No natural enemies of DBM have been recorded (R. Weller pers. comm. 1985).

Western Samoa

An unidentified wasp occasionally attacks DBM larvae (T.V. Bourke pers. comm. 1986) but no surveys for parasitoids or predators have been carried out (I. Aloalii pers. comm. 1990).

Discussion

The biological control of DBM in the Pacific is complicated by the fact that, in many locations, it is only one of a group of introduced brassica pests, most of which need to be controlled. This means that, even if DBM came to be heavily attacked by natural enemies, to the extent that it was no longer a problem, insecticides would still have to be applied to the crop. It follows, therefore, that consideration should be given to one or more of three approaches:

(i) to embark, preferably simultaneously, upon the biological control of at least the most important pests associated with DBM, and in particular of *C. pavonana* and *Hellula* spp. (Table 1);
(ii) to encourage the use of sprays that are effective against the brassica-attacking Lepidoptera, but relatively harmless to parasitoids (e.g. *B. thuringiensis* preparations or neem); and
(iii) to select, or otherwise develop, strains of parasitoids resistant to the harsher insecticides that it is desired to use. However, although some field resistance in parasitoids is reported (e.g. Iman et al. 1986) this approach is likely to prove of limited value because of the capacity of DBM to develop resistance rapidly to harsh insecticides used for any length of time.

In relation to the biological control of *C. pavonana*, its natural enemies in the oceanic Pacific are listed in Table 4. It is interesting that *C. pavonana* occurs, but is regarded as only occasionally important, in Western Samoa, New Caledonia and French Polynesia (Table 1). The reasons for its relatively low status in these regions are worthy of investigation: no records of parasitoids from these countries are available. *Crocidolomia* larvae were rare and DBM larvae absent from cabbage during a visit to Tau Island (American Samoa) in 1986. An *Apanteles* sp. was abundant,

Table 4. Natural enemies of *Crocidolomia pavonana* in the Pacific.

Country and natural enemy	Family	Stage attacked	Reference
Cook Islands			
none found			Simmonds 1971
Fiji			
Apanteles sp.	Braconidae	larva	Lever 1944
Chelonus sp.	Braconidae	larva	Lever 1944
Papua New Guinea			
Exochus sp.	Braconidae		Greve & Ismay 1983
Apanteles sp.	Braconidae	larva	Gagne 1979
a braconid		larva	Gagne 1979
Brachymeria sp.	Chalcididae	pupa	Gagne 1979
Diadegma sp.	Ichneumonidae		Greve & Ismay 1983
? *Xanthopimpla* sp.	Ichneumonidae	pupa	Gagne 1979
Ropalidia bambusae	Vespidae	larva	Gagne 1979
Richards		(predator)	
tachinid fly	Tachinidae	larva	Thistleton 1979
Palexorista solennis Walker	Tachinidae	larva	Greve and Ismay 1983
P. inconspicuoides (Baronov)	Tachinidae	larva	Baranov 1934
Palexorista sp.	Tachinidae	larva	Gagne 1979
American Samoa			
Apanteles sp.	Braconidae	?	P. Maddison pers. comm.
Tonga			
an ichneumonid	Ichneumonidae	?	SPC 1979, Crooker 1979

presumably a parasite of *Crocidolomia*, which was causing very little damage (P.A. Maddison pers. comm. 1986).

There do not appear to have been any attempts at biological control of *C. pavonana*, a native of Africa and Asia. More than 20 larval parasitoids are known, most from the Indian subcontinent (Waterhouse and Norris 1987). A 5 year search was initiated in 1978 for agents in Pakistan, resulting in reports of an *Apanteles* sp. (*ultor* group) (CIBC 1981) and an unidentified braconid wasp and a tachinid fly (CIBC 1982). Since *C. pavonana* is reported to be a widespread and, from time to time, particularly where pesticides are used, an important pest in its native range, the prospects for finding effective biological control agents do not appear to be good. However, existing knowledge is rather limited and it is possible that, if freed from their hyperparasitoids, its parasitoids might be far more effective in a new country. Furthermore it is said that the current use of pesticides greatly diminishes the effectiveness of the parasitoids that are present. The species is certainly worthy of more detailed investigation.

Information on the possibilities for biological control of the cabbage pests *H. undalis* and *H. rogatalis* is summarized by Waterhouse and Norris (1989) and for *H. armigera* and *S. litura* by Waterhouse and Norris (1987).

DBM is attacked by more than 90 parasitoids (Goodwin 1979). Unfortunately, many of these appear to cause only low levels of mortality. In their native range some are heavily attacked by hyperparasitoids. It is beyond the scope of this paper to attempt to evaluate the relative importance of the parasitoids dealt with by a number of authors, including Lim (1986a), Lloyd (1940) and Chua and Ooi (1986).

In general, egg parasitoids (belonging it seems exclusively to the genera *Trichogramma* and *Trichogrammatoidea*) appear to contribute little. Larval parasitoids have the greatest control potential, especially those belonging to the genera *Diadegma*, *Cotesia* and *Apanteles*. Of these,

D. semiclausum, *D. insulare* and *C. plutellae* appear to be the most promising for the oceanic Pacific. Recent work suggests that *C. plutellae* may be a complex of at least two species and that DBM is attacked by at least six 'biological' species of *Diadegma*, some of which are almost impossible to separate using traditional morphological characters (M.G. Fitton and A.K. Walker pers. comm. 1990). This may explain why varying results have been reported by different workers. It may also explain unsuccessful attempts to establish, in warm lowland areas with temperatures approaching 30°C, the 'strain' of *D. semiclausum* that is effective in the cool highland areas of Malaysia and Taiwan (N.S. Talekar pers. comm. 1990). Of the pupal parasitoids, *D. collaris* and *O. sokolowskii* should not be overlooked. Also, if inoculated widely into the brassica environment, it is possible that the fungus *Zoophthora radicans* would continue to cause heavy mortality when weather conditions were suitable.

Of the parasitoids, laboratory experiments suggest that *D. semiclausum* should be superior to *D. collaris*, with *C. plutellae* in third position. However, in the field, *C. plutellae* performed best (Lim 1986a; Chua and Ooi 1986). Furthermore, *C. plutellae* is reported to attack larvae of *C. pavonana*, *H. undalis* and *Agrotis ipsilon*, although the level of attack was very low in the field (Lim 1982, 1986b).

It is clear from the very sparse distribution in Oceania of introduced parasitoids (Table 2) that much scope still exists for increasing overall levels of parasitization in all countries, including both Australia and New Zealand. Even if they were unable to reduce DBM to the status of a minor pest under all circumstances, the impact of a complex of parasitoids is likely to be of great benefit, particularly to the relatively insecticide-free environment of traditional farmers, and certainly merits exploitation.

It is clear that any success with biological control of DBM (and other cabbage pests) is most appropriately viewed as a major step towards an integrated pest management package, which should also involve, as appropriate, other measures such as selection of sprays that are minimally harmful to parasitoids, plant resistance, overhead water sprays, provision of flowering plants to supply nectar to boost parasitoid reproduction, and the use of trap crops. There have been many reports in recent years of interplanting or trap cropping. (e.g. Waterhouse and Norris 1987), but none as promising as those recently of Srinivasan and Krishna Moorthy and also of Muniappan and Marutani (this workshop). They found that a trap crop of earlier-planted mustard or radish diverted most or all *Crocidolomia* and *Hellula*, from head cabbage.

In much of the oceanic Pacific (and particularly in traditional agriculture) where insecticide use is still far lower than in many other regions, and where the ultimate in unblemished appearance of a consumer product is not demanded, biological control of DBM, and hopefully of associated pests, holds considerable promise. Great care should be taken with any introductions to avoid hyperparasitoids (of which there are many) and indeed any organisms other than the intended agents.

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Hymenopterous Parasitoids Associated with Diamondback Moth: the Taxonomic Dilemma

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Abstract

Attempts to control diamondback moth *Plutella xylostella* (L.) using insect parasitoids have not been entirely successful. Parasitoids which have been utilized include *Diadegma* species and *Cotesia plutellae*. A better understanding of the systematics of these Hymenoptera could lead to their more effective exploitation in biological control. *Diadegma* is a very large and difficult genus of Ichneumonidae. There are no completely satisfactory taxonomic treatments, and from the limited work that has been done we know that some distinct biological species are almost impossible to separate using traditional, morphological characters. Nine putative species of *Diadegma* attack diamondback moth. So far no studies have adequately considered the taxonomic questions which are important in relation to their parasitism of this widespread pest. The microgastrine braconid *Cotesia plutellae* has been used with limited success in controlling diamondback moth, but recent field studies have raised suspicions that it is a complex of two or more species. We present a review of our knowledge of *Diadegma* and *Cotesia* and other microgastrines associated with diamondback moth, and attempt to outline a strategy for solving the taxonomic problems, leading to a better understanding of relationships with this host. The other parasitoids which we consider reliably recorded from diamondback moth are also noted.

Introduction

This paper concentrates on *Diadegma* and *Cotesia*, but briefly touches on other hymenopterous parasitoids of diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera; Yponomeutidae). In each of these three sections, the current taxonomic situation, related questions of biology, and strategies for the future are considered.

Diadegma

Aspects of *Diadegma* Taxonomy

Basic morphotaxonomy. The 200 valid, described species of *Diadegma*, probably make up less than half the real total of this large, worldwide genus. Its taxonomy is fraught with difficulties and there are very many misidentifications in the literature. No completely satisfactory treatments (with keys, descriptions and summaries of biologies and distributions) exist, even for limited faunas. In Europe, where over 120 species are recognized, many additional, 'cryptic' species undoubtedly await detection. For example, the morphospecies known as *D. chrysostictos* has been shown by Horstmann and Shaw (1984) to comprise two species which attack different hosts in different habitats, but which are both so similar and so variable as to make impossible the reliable identification of many individual specimens.

The species associated with DBM. As far as the *Diadegma* species associated with DBM are concerned, the situation is particularly confused because poor communication, and nomenclatural and identification problems have added to the taxonomic difficulties. For example, as recently as 1974 and 1988 new species of *Diadegma* parasitizing DBM were described with no reference to *D. semiclausum*, of which they could well turn out to be synonyms.

Although there are some published data, for example, in Venkatraman (1964), no studies convincingly link distinguishing morphological characters to demonstrably separate biological species. Even in Europe, where two species, *D. semiclausum* and *D. fenestrale*, have been recognized as parasitizing DBM, many questions remain unanswered. As long ago as 1938, Hardy reported the successful production of hybrids in the laboratory and suggested that interbreeding between these species might explain the occurrence in the field in England of 'all stages of intermediate forms.'

The host, DBM, is cosmopolitan, its geographical origin is uncertain and it could be attacked by 'local' *Diadegma* species as well as those which have had a long association with it. In both New Zealand (Muggeridge 1930) and South Africa (Ullyett 1947) supposedly native species of *Diadegma* were reported as parasitizing DBM before the introduction of *Diadegma* species from Europe.

Traditional taxonomic studies have been hampered partly because numbers of specimens in collections are low. Voucher series relating to many of the transfers, introductions and laboratory studies are inadequate or even nonexistent. Much more material is needed for proper investigations.

The present confused taxonomic situation. No-one has made a special study of the *Diadegma* species associated with DBM. The annotated list below summarizes the present situation: it is for guidance only and it is not meant to imply that there are nine separate biological species of *Diadegma* attacking DBM. The species are in alphabetical order and the currently accepted synonymy (indented) follows the valid species name.

Diadegma fenestrale (Holmgren, 1860)

gracilis (Gravenhorst, 1829)

This European species probably has *Cnephasia stephensiana* (a tortricid which feeds on a variety of herbaceous plants) as its main host, but is able to move on to DBM in suitable situations. Available information suggests that it may not be able to sustain populations on DBM in the absence of its usual host. Lloyd (1942), for instance, found it difficult to get females to oviposit in DBM larvae. It may interbreed with *D. semiclausum* (Hardy 1938).

Diadegma insulare (Cresson, 1865)

polynesialis (Cameron, 1883)
hellulae (Viereck, 1912)
plutellae (Viereck, 1912)
pygmaeus (Viereck, 1925)
congregator (Walley, 1926)

This New World species (Carlson 1979) is recorded from southern Canada south to Venezuela and west to Hawaii, and from the pyralid *Hellula undalis* and *Plutella armoricae* as well as DBM. Most of the synonymy shown above seems to be the result only of examination of dry pinned material. It needs to be critically reassessed. The wide geographical distribution and the early dates of description of some the synonymized species begs the question of the real number of species involved. Even if there is only one, potential for use in biological control may vary considerably between populations. *D. plutellae* has sometimes been recognized as

a distinct species (for example, by Gupta 1987) but without any justification or characters for its separation being given. There have been few attempts to introduce *D. insulare* to new areas to control DBM.

Diadegma leontiniae (Brèthes, 1923)

This species was described from specimens reared from DBM in Argentina and has subsequently been recorded (also from reared material) from Uruguay. It may be the *Diadegma* species reported from DBM in Chile (Rojas, 1965). It may also be a synonym of *insulare* but (see discussion above) no-one seems to have considered that possibility.

Diadegma rapi (Cameron, 1912)

D. rapi from Australia is recognized easily because it lacks one small cross-vein in the forewing. There seems to be reproductive isolation of this species (Venkatraman 1964). There are no records of hosts other than *P. xylostella*, although no effort appears to have been made to find any. It has been moved within Australia to help control *P. xylostella* (Wilson 1960), but was outperformed by *D. semiclausum*.

Diadegma semiclausum (Hellén, 1949)

eucerophaga Horstmann, 1969
cerophaga – misidentification
tibialis – misidentification

This is the best-known of the *Diadegma* which parasitize DBM. After 1969 the name *eucerophaga* quickly came into general use, but in 1980 Horstmann synonymized it with *semiclausum* (a species described from the Azores) and unfortunately this name change has taken a long time to get into the literature relating to DBM. *D. semiclausum* seems originally to have had a Eurasian distribution but it has been widely introduced for biological control. However, the identity of material used for introductions requires further investigation (see below).

Diadegma varuna Gupta, 1974

Described from India, compared in the original description to *fenestrale*, but not *semiclausum*. It may be a synonym of *semiclausum*.

Diadegma xylostellae Kusigemati, 1988

Described from a single male from Nepal. Said to be very close to *D. varuna*.

Diadegma sp. indet. A.

lateralis – misidentification

This is the species, supposedly native, which was found attacking DBM in New Zealand before the introduction of *Diadegma* species from England (Muggeridge 1930).

Diadegma sp. indet. B.

This is the species, supposedly native, which was found attacking DBM in South Africa before the introduction of *Diadegma semiclausum* from Europe (Ullyett 1947). Ullyett reported that the two species interbred.

Biology of *Diadegma*

Some aspects of the biology of *Diadegma* deserve attention because they are important in relation to both taxonomy and biological control. Their significance has been overlooked because of the confused taxonomic situation.

Host range. Data in the literature suggest that many *Diadegma* species have wide host ranges. For example, Hardy (1938) apparently accepted that *D. fenestrale* attacked 24 species of Lepidoptera and one coleopteran, describing it as 'very polyphagous'. Although such lists do include accurate records, many of the records need verification and they are potentially extremely misleading (see, for example, Shaw 1981). Critical appraisal of what is now known of the patterns of host associations and the mechanisms involved (see, for example, Dijkerman 1990) leads us to conclude that most species of *Diadegma* are relatively host-specific. The usual hosts are microlepidoptera. For example, *D. fabricianae* is essentially restricted to *Anthophila fabriciana*, a choreutid moth which feeds on nettles (Horstmann and Shaw 1984). *Diadegma* species which undoubtedly have more than one host in nature often have some common factor linking them. *D. chrysostictos* is such a species, parasitizing a small number of pyralid moths which live in a narrowly defined niche, in this case basic human foodstuffs (Horstmann and Shaw 1984). The host ranges of *D. fenestrale* and some other species recorded attacking DBM are undoubtedly, similarly circumscribed. One species of *Diadegma* is suspected to alternate between a micro and a macro-lepidopteran feeding on the same plant, but so far the evidence for this is circumstantial (Fitton and Shaw, unpublished data).

Annual cycles and 'migration.' It is not known how the *Diadegma* species associated with DBM overwinter in temperate zones. The most common method of overwintering in ichneumonids is as a diapausing, full-grown larva within its own cocoon (which may be within some host remains, such as a pupa). In multivoltine species sometimes only a proportion of late summer generations enter diapause, the remainder survive or perish depending on weather conditions. In many crop situations *Diadegma* cocoons might be destroyed during winter plowing but other crucifers could possibly harbor a large enough overwintering population to explain the sometimes high rate of parasitism of the spring generations of DBM. A better knowledge of the overwintering mechanism of the *Diadegma* is essential in relation to biological control.

It is well known that DBM is migratory. No-one seems to have considered that some of its parasitoids might also be migratory. One small piece of evidence supporting the idea of annual reinvasion of parts of the host's range by *Diadegma* is Putnam's (1978) conclusion that *D. insulare* does not survive over winter in Saskatchewan, where it is one of the two major parasitoids of DBM. Because the moth overwinters as an adult some workers have jumped to the conclusion that *Diadegma* must overwinter in association with alternate host species.

Habitats. Little attention has been paid to the habitat requirements of adult *Diadegma*. Adults of the subfamily Campopleginae, to which *Diadegma* belongs, have been observed feeding from flowers (Fitton and Jervis, unpublished data) and the availability of suitable flowers in and around crops could influence levels of parasitoid activity in the crop itself.

Biosystematics and Biological Control

Biological differences between populations (whatever the taxonomic status of the populations) can be of crucial significance in biological control and need to be explored thoroughly if maximum benefits are to be expected from attempts to control DBM using *Diadegma*. In the case of *Diadegma* overreliance on identifications and taxonomic decisions made by museum workers has probably restricted the approach of biological control researchers.

How many species? Reports in the literature of copulation between some of the different 'species' of *Diadegma* associated with DBM; successful rearing of offspring of both sexes from such pairings; and of morphological intermediates should all lead to questioning of the species defined by taxonomists working only with limited museum material. Field biologists are usually in a far better position to make observations which will lead to recognition of real biological species by taxonomists. Properly integrated studies on *Diadegma*, involving museum taxonomists and field workers, should give a far clearer picture and better understanding, from which can flow better-directed investigations of populations for utilization in biological control.

The major questions relate to:

(1) The relationships of the *Diadegma* 'species' attacking DBM. Can morphological characters be found? Do they all belong to one species-group? Is there biological variation within 'morphospecies'?

(2) The extent of interbreeding. Has it occurred and what have been the consequences? Could interbreeding be exploited for biological control?

(3) Hosts other than DBM. What are the host ranges in nature, and how are they circumscribed?

Founder effect and changes in introduced populations. The subject of what happens to founder populations is currently attracting attention. Population geneticists have theorized extensively on changes in species after they have been introduced into new areas but few hard data are available. The possibilities of obtaining information are increasing with the introduction of 'DNA-methods' and the refinement of serological techniques. Biological control provides some ideal systems, and *Diadegma*-DBM is one, for investigation.

Genetic change in organisms is of particular interest to the practice of biological control. Much effort is spent in carefully matching hosts and climates and phenologies in the selection of populations of agents for introduction in control programs and in maintaining diversity in cultures. A project based on *Diadegma* populations associated with DBM could bring results of practical and academic value.

The material of *Diadegma* introduced from England to New Zealand in 1936 undoubtedly included *semiclausum* and *fenestrale*. As New Zealand has been the source of much of the so-called *D. semiclausum* used elsewhere, how does the population in New Zealand differ from the present one in Europe? Did any *fenestrale* survive? Did any hybrids between the two species have an effect on the gene pool of the newly established population? Did the newly introduced *Diadegma* interbreed with the supposedly native species already parasitizing DBM?

Future studies. A first priority must be to establish the kind, range, and geographical distribution of variation. The project should involve investigation of morphological, enzymatic and genetic characters.

(1) Detailed morphological studies and morphometric analyses could be undertaken on more-or-less all the populations involved. Material, particularly reared specimens, in series of 100+, of both sexes, preserved in 95% alcohol (for preference), as well as pre- and post-release samples with full data would be essential. This should enable some of the basic taxonomic questions to be answered and others to be formulated more precisely.

(2) Analysis of enzyme systems using electrophoresis on selected populations would allow a wider range of initial comparisons than the DNA investigation suggested below and should help solve some of the more difficult taxonomic problems. Cultures could be obtained from different parts of the world, and maintained in quarantine, to provide the necessary fresh material.

(3) Selected DNA investigations. Analyses of isoenzymes and DNA sequence variations could provide means for characterizing cryptic species. Comparison of *Diadegma 'semiclausum'* from New Zealand and England would probably produce the most informative results first.

(4) The laboratory cultures could be used to obtain data, e.g. on temperature tolerance, which would help match populations to climatic and environmental conditions.

(5) Experimental interbreeding of different populations would yield information on the degree of reproductive isolation between populations and 'species.'

(6) Field investigations would be needed to determine modes of overwintering and host ranges in nature.

Cotesia

Cotesia plutellae (formerly *Apanteles plutellae*) does not have a complicated taxonomic history. It belongs to the very large braconid subfamily Microgastrinae. The species was originally described in 1912 by Kurdjumov from material reared from DBM. However, until Wilkinson (1939) redescribed the species in detail and pointed out differences from, and its closeness to, the well-known cosmopolitan species *C. ruficrus*, it was not properly recognized. Very recently it has been suggested that *plutellae* may be a junior synonym of *vestalis* Haliday, but this requires further investigation. Another slight complication is that many new genera have recently been recognized in the subfamily Microgastrinae, with the result that many species previously in *Apanteles* have new generic assignments. However, the new classification has not gained universal acceptance and continuing use of *Apanteles* sensu lato by some workers is causing confusion.

Eighteen related species of microgastrine braconids are recorded from DBM in the literature. Many of these are probably misidentifications of *Cotesia plutellae* but all relevant material needs to be reexamined and investigated further. The North American species *Microplitis plutellae* is already verified and considered as reliably recorded from DBM. It has been found destroying around 10% of host larvae in the final instar (Harcourt 1963). *Apanteles ippeus*, widely distributed in eastern Australia (Yarrow 1970; Hamilton 1979), is only recorded from DBM, but its biological control potential seems to have been overlooked. *Apanteles ippeus* is not closely allied to *Cotesia plutellae*; it belongs to *Apanteles* sensu stricto.

Cotesia plutellae has been introduced into many countries and helps control DBM. Until recently no-one questioned its taxonomic status. However, Andreas Poelking (pers. comm.), working in the Philippines, discovered that there is a better survival rate in a Taiwan strain than a Philippines strain and suspects that there are two different species involved. One of us (AKW) has examined his material of both 'strains' but can find no consistent morphological differences of the kind currently used for separation and recognition of microgastrine species. However, further morphometric analysis (Poelking, unpublished data) has revealed differences which await further study.

Another aspect of *C. plutellae* which deserves critical appraisal is its host-specificity. Contrary to popular assumption it may not be host-specific. It is recorded from 20 species of Lepidoptera, the majority of the records relating to north-west Europe. Several questions need to be asked immediately. Are other 'cryptic' species of *Cotesia* confused with *C. plutellae*? How reliable are the records? What bias is there in the records. What is the real host range of *C. plutellae* and what are the consequences for biological control of DBM?

Other parasitoids of DBM

Some consideration of the other parasitoids is worthwhile. About 50 species are recorded by Thompson (1946) as attacking DBM. Such compilations are prone to error (Shaw 1981) and the real total of regular parasitoids is probably a fraction of this number. Recent reviews (such as those of Lim 1986) lack a critical taxonomic component.

Ichneumonidae other than Diadegma. The ichneumonine *Diadromus collaris* (formerly in *Thryraeella*) attacks the pupae of DBM and has been widely introduced in biological control attempts. Other ichneumonid primary parasitoids include a second species of *Diadromus* belonging to another species-group, *D. subtilicornis*, and the metopiine *Macromalon orientale*. There appear to be no taxonomic problems associated with the foregoing. However, the identification and role of ichneumonid hyperparasitoids has been more confused. Mesochorines (identified as *Mesochorus*) are obligate, internal, larval hyperparasitoids of braconids and other ichneumonids. In Asia a phygadeuontine, *Diatora* species (often misidentified under various names including *Hemiteles*), is a pseudohyperparasitoid which lays its eggs in the cocoons of *Cotesia plutellae* (and possibly also of *Diadegma* species). A member of a related genus (so far unidentified)

with a similar biology occurs in the West Indies. Species of *Itoplectis* (subfamily Pimplinae) may be primary, or facultative or obligate hyperparasitoids.

Braconidae other than Microgastrinae. Five other braconids, belonging to the genera *Meteorus*, *Bracon* and *Chelonus*, have been recorded from DBM, but until they are investigated further the records should be treated as doubtfully correct.

Chalcidoidea. About 30 species of Chalcidoidea have been recorded in the literature as primary or hyperparasitoids of DBM. However, many of these records are from rather dubious, single casual rearings and require verification from additional rearing to be considered as reliable.

The eulophid *Oomyzus sokolowskii* (formerly in *Tetrastichus*) is a primary and facultative hyperparasitoid and it is the only chalcidoid to have shown any real potential for biological control of DBM, and it has been introduced into many countries. Rates of parasitism have been recorded as high as 89-100% for DBM (Cock, 1985), and levels of hyperparasitism through *Cotesia plutellae* as high as 10%. Although it can act as a hyperparasitoid, Waterhouse and Norris (1987) expressed the opinion that it might still prove to be effective in biological control, pointing out May and Hassell's (1981) work which indicated that facultative hyperparasitoids could be thought to interact in the same way as two competing primary parasites and not adversely affect the system.

Tetrastichus howardi (also known as *T. ayyari* and *T. israeli*) is a common parasitoid of a variety of Lepidoptera in Southeast Asia, including DBM. However, it has not been shown to be an effective biological control agent against any of them. Several species of *Trichogramma* have been mentioned but their taxonomy is fraught with difficulties and species names need to be regarded with caution. Effective control by *Trichogramma* species is rarely achieved except through extensive inundative release programs. Several species of *Spilochalcis* and *Brachymeria* have been reared, but none appear to show promise as control agents and some may even be acting as facultative or obligate hyperparasitoids. Other species of chalcidoids mentioned as primary parasites need verification. Species in the genera *Trichomalopsis* (= *Eupteromalus*), *Elasmus* and *Mokrzeckia* which have been mentioned may well be hyperparasitoids.

It appears that detailed taxonomic studies of Chalcidoidea will not be worth pursuing on a cost-effective basis in looking for biological control agents for DBM At some point it may be worth studying the whole complex of hyperparasitoids, but they should not be considered to be high on any list of priorities at the present time.

Other superfamilies. The genus *Ceraphron* of the superfamily Ceraphronoidea is mentioned in the literature in association with DBM. Cock (1985) records *Aphanogmus fijiensis* (formerly in *Ceraphron*) as a hyperparasitoid of *Cotesia plutellae* in Barbados, with rates as high as 13%.

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Diamondback Moth and its Natural Enemies in Jamaica and Some Other Caribbean Islands

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Abstract

Diamondback moth (*Plutella xylostella* (L.)) is widely distributed in the Caribbean. In Jamaica, amongst 14 pest species feeding on cabbage, cauliflower and other crucifers, diamondback moth, cabbage white butterfly and armyworms inflict the highest damage. The combined crop damage from these pests ranges from 74 to 100%, averaging 79%. Of these, diamondback moth alone represents over 75% of the pest populations, causing up to 90% crop loss. The highest (February 1989) and lowest (August 1989) populations per plant were 43 and 1 larvae in Douglas Castle and 63 and 2 larvae, respectively, in Castle Kelly areas. In Jamaica, five parasite species, viz. *Trichogramma* sp., *Diadegma insulare*, *Cotesia* (= *Apanteles*) sp. (*glomeratus* group), *Oomyzus* (= *Tetrastichus*) *sokolowskii* and *Trichospilus diatraeae* were found parasitizing different developmental stages of the pest. Additionally, *Coleomegilla maculata*, *Cycloneda sanguinea*, *Toxomerus dispar*, *Toxomerus watsoni* and *Pseudodorus clavatus*; *Ceraeochrysa claveri*, and *Belonuchus gagates* were preying upon them. The fungi *Beauveria bassiana*, *Hirsutella* sp. and *Paecylomyces* sp. were found infecting larvae and pupae in the plains and sub-mountain areas. During March 1989, a larval parasite *Cotesia plutellae* was introduced from Barbados. Soon after its release at the University of the West Indies Mona Campus and Bodles Agricultural Experimental Station, it was recovered. Between March 1989 and July 1990, the levels of parasitism at Bodles, ranged from 5.4 to 88.7% (average 51%). As a result of high mortality caused by *C. plutellae*, the diamondback moth populations were reduced significantly. Since the establishment of *C. plutellae* in Jamaica, four species of hyperparasites, *Aphanogmus* (= *Ceraphron*) *fijiensis*, *Horismenus* sp. (a new species), *Catolaccus* sp. (a new species) and *Spilochalcis* sp. (a new species) were found attacking the cocoons. *Spilochalcis* sp. also attacks the cocoons of *D. insulare*.

Introduction

Cabbage (*Brassica oleracea* var. *capitata*) , cauliflower (*Brassica oleracea* var. *botrytis*) and other cruciferous crops are important in the diet of Caribbean people. Cabbage is the most widely grown in Jamaica, fetching high market prices (US$0.22-0.68/kg). Between 1973 and 1986 cabbage production increased significantly (from 6985 to 15,150 tons) (Ministry of Agriculture, unpublished reports for 1973 to 1986). The potential for these crops is, however, limited because of insect pests.

Out of 14 insect species feeding on crucifers, the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), is the most important. In Jamaica as well as other parts of the world, when insecticides are not used, crop losses caused by DBM may reach 50-100% (Calderan and Hare 1986 and Sagenmuller and Rose 1986). Because of its high reproductive capability and its ability to develop pesticide resistance quickly, it has become less susceptible to conventional insecticides (Ankersmit 1953, Calderon and Hare 1986, Lim and Khoo 1986; Miyata et al. 1986, Morallo-Rejesus 1986; and Suah and Ellis 1974).

In Jamaica, insecticides recommended since 1968, and now ineffective, include malathion, galecron, gardona, dipterex, diazinon, carbaryl, surecide, DDT, belmark and deltamethrin (Suah and Ellis 1974), whilst Thuricide, Biotrol, permethrin, methomyl and profenofos are of questionable reliability.

The general pest management and environmental, occupational and consumer health problems created by insecticides have forced the field entomologists to put greater reliance on biological control. In the Caribbean, the Ministry of Agriculture Barbados and the Commonwealth Institute of Biological Control (CIBC) (now International Institute of Biological Control (IIBC)) Trinidad, West Indies, initiated research on the biological control of DBM in the 1970s and was later extended to the eastern Caribbean islands by the Caribbean Agricultural Research and Development Institute (CARDI) (Alam 1974, 1982; Bennett and Yaseen 1972). This report deals with research done in Jamaica with brief mention of the status of natural enemies in other Caribbean islands.

Methodology

The evaluation of the insect pests and their natural enemies was done monthly. Field visits were made to a number of farm sites in different parishes in Jamaica. For more regular observations, fields were selected in three ecological zones; Castle Kelly (plains: elevation 457 m), Douglas Castle (610m) and Blue Mountain (hills : 1219 m) (Fig. 1). Besides their ecological differences, these are major cabbage-growing areas. Trial plots were also established at Bodles Agricultural Experimental Station.

The following sampling technique was adapted: From a 0.04 ha field five cabbage plants were taken at random and brought to the laboratory for detailed examination. The plants were weighed, every leaf examined and all stages of the pest(s) recorded. Eggs, larvae and pupae of the pests were isolated in glass vials and kept to rear out their natural enemies or the adult stage.

Data on the amount of damage caused to each cabbage head by various insect pests, and their relative abundance, were measured on a rated scale. The parasites reared from immature stages of the pests (i.e. eggs, larvae and pupae) were recorded and their levels of parasitism calculated. Adults and immature stages of predatory insects and pathogens collected in the field were also recorded. Information on hyperparasites attacking primary parasites was gathered. The biology of important parasites was studied in the laboratory.

Fig. 1. Areas of observation for DBM studies in Jamaica.

Results

Fourteen species of insect pests and three species of slugs were found feeding on cabbage, cauliflowers and other crucifers. The most important of these were the DBM, cabbage looper (CL) (*Trichoplusia ni* Hübner), armyworms (*Spodoptera frugiperda* (J. E. Smith) and *Spodoptera latifascia* (Walker), (Lepidoptera: Noctuidae), cabbage white-butterfly [*Ascia monuste* (L.), Lepidoptera: Pieridae] and cabbage aphid [*Brevicoryne brassicae* (L.), Hemiptera: Homoptera: Aphididae]. In the Blue Mountain areas, besides these pests, a pyralid [*Evergestis rimosalis* (Guenee), Lepidoptera:Pyralidae] was found damaging the crop. In Douglas Castle three species of slugs *Leidyula portoricensis*, *Leidyula* sp. and *Sarasinula plebeia* (Veronicellidae) caused heavy crop losses.

Pest Status

Alam (1982) reported that in the Caribbean where crucifers are grown throughout the year, and conditions exist for continuous breeding of DBM which causes serious crop losses to farmers, the application of insecticides at short intervals (two or three applications/week) resulted in the development of insect resistance to some chemicals (Alam 1974).

In Jamaica, the combined crop damage caused by various defoliators ranges from 74 to 100%. Of these, DBM represents over 75% of the pest population, causing up to 90% of the crop damage. When the DBM population is reduced, it gives way to the build-up of other defoliators, particularly CL.

Distribution and population levels of DBM in Jamaica

Between March 1988 to July 1990, irrespective of the ecological variations, DBM was equally important in the plains, sub-mountains and hilly areas. Peak population varied for each area.In Castle Kelly the highest number of DBM was recorded in February 1988, June 1989 and February 1990, in Douglas Castle in April and July 1988, February, May and December 1989 and January and February 1990 and in Blue Mountains in May and September 1989 with a moderate peak in July 1989.

Generally the population tended to be low in August and October in all observation sites. In Castle Kelly, the lowest number of DBM was recorded in March, May, August and November 1988, May and July to December 1989, and May 1990. In Douglas Castle it was in August 1988 and August to October 1989, and in Blue Mountains in June, August and October 1989. Larval population per plant also varied. The maximum number of DBM larvae per plant recorded in Castle Kelly and Douglas Castle was 63 and 43 respectively in February 1989, and the minimum of two larvae/plant from the former and one larva/plant from the latter locality in August 1989. In Blue Mountains 38 larvae/plant were collected in May and 23 larvae/plant in June 1989. At Bodles Agricultural Experimental Station the sample populations from each field investigation varied from 34 to 518 larvae per month, with an average of 229 larvae/month for the period April 1989 to March 1990.

Natural enemies

In spite of heavy use of chemical insecticides against DBM and other defoliators on cabbage and cauliflowers in the Caribbean, a number of natural enemies remain active in the fields, providing significant control.

Alam (1974, 1982) reported *Trichogramma* sp. (Hymenoptera:Trichogrammatidae), *Cotesia* (= *Apanteles*) sp. (*ater* group) (Hymenoptera:Braconidae:Cotesiini) and *Spilochalcis hirtifemora* (Ashmead) (Hymenoptera:Chalcididae) attacking eggs, larvae and pupae of DBM, respectively, in Barbados. Besides these, three species of coccinellids, seven of ground beetles (Carabidae), two of 'Jack Spaniard' wasps (Vespidae), three of ants (Formicidae), two of lacewing

bugs (Chrysopidae) and one each of earwig (Carcinophoridae) and centipede (Scolopendridae) were found preying upon the pest(s). Yaseen (1974) reported *Cotesia* sp. (*ater* group) and *S. hirtifemora* from Trinidad. A similar complex of natural enemies was also found in the eastern Caribbean islands (Alam, CARDI's unpublished annual and visit reports, 1977 to 1990; Table 1).

Pest/parasite relationship

The variability of the populations of pest and parasite is shown in Fig. 2. The data for each insect have been standardized to have zero mean and unit variance. This was done because the pest data are of much higher values than the parasite data, so to plot both on the same graph is not helpful. It is these standardized observations that have been plotted against the cumulative frequency distribution and against time. The cumulative frequency distributions (Fig. 2) attempt to show how similar the populations of the pests and parasites are. Although they cannot be regarded as being statistically similar (Kolmogarov test, $P > 0.05$), there does seem to be a strong degree of similarity which is hard to ignore. Kendal's rank correlation coefficient, which makes no assumption as to the underlying distribution of the data, is significant ($P < 0.001$) for each pair of pest and parasite illustrated, from which one would infer that the two variables are associated from the populations sampled.

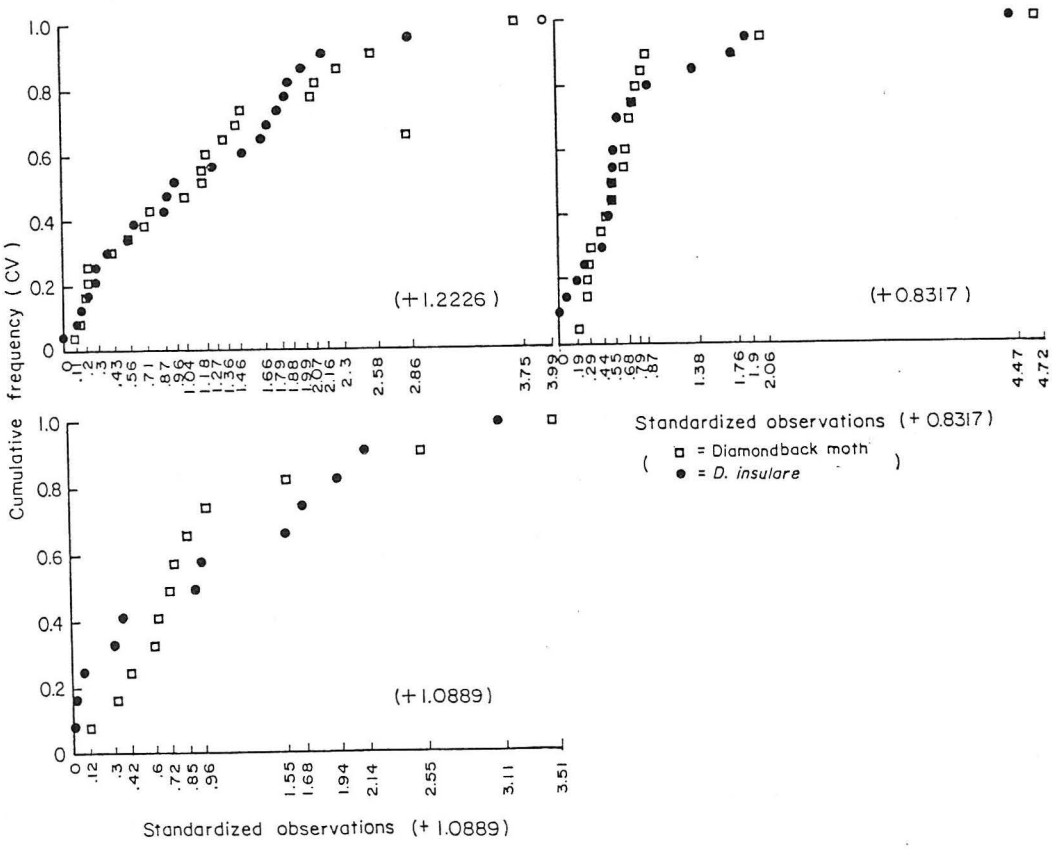

Fig. 2. Cumulative distribution of DBM and *D. insulare* at Douglas Castle from March 1988 to July 1990.

Indigenous parasites

Diadegma insulare (Cresson)

Distribution and nature of parasitism: *Diadegma insulare* is the most common larval parasite of DBM in Jamaica, and remains active throughout the year. The female oviposits in 2nd and 3rd instar larvae. The parasite larva feeds internally without killing the host, until the host larva spins a thin silken cocoon for pupation. At this stage the parasite (*Diadegma*) grub consumes the host (DBM) larva completely and spins an oval shaped cocoon of its own for pupation.

Levels of parasitism: The populations of *D. insulare* fluctuate with the density of DBM (Fig. 2). In Douglas Castle area, the levels of parasitism ranged from 12 to 28.5%, between April and December 1988, 6.1-75.8% from February to December 1989 and 7.9-46.4% from January to July 1990. The cocoons of *D. insulare* were attacked by a hyperparasite *Spilochalcis* sp., parasitizing 0 - 67.3% in 1988 and 0 - 50% in 1989.

In Castle Kelly (Fig. 2) during March, May, August and November 1988, the levels of parasitism ranged from 9.4 to 45.5%, from February to December 1989 0 - 41.8%, and from January to July 1990 0 - 45.5%. *Spilochalcis* sp. attacked 8.3-68.6% cocoons of *D. insulare* in 1988, and 0-25% in 1989.

In Blue Mountains, the levels of parasitism by *D. insulare* ranged from 1.1 to 57.4%, average 13.8%, between April and November 1989. *Spilochalcis* sp. was recorded only in August and September 1989, attacking 83.3 and 1.6% of the cocoons of *Diadegma*, respectively.

At Bodles Agricultural Experimental Station (Fig. 2), *D. insulare* was rare. Only 2.6% parasitism was recorded in July 1989 and 2% in March 1990. Forbes and Mansingh (1990) reported 21.5-62% parasitism by *D. insulare* in Douglas Castle, except in August and October 1987. At Sandy River the levels of parasitism recorded were 8.3% in May to 55.9% in September 1986.

Oomyzus (= *Tetrastichus*) *sokolowskii* (Kurdjumov)

Distribution and nature of parasitism: This gregarious larval-pupal parasite of DBM is indigenous to Windward and Leeward islands of the Caribbean. In Barbados it was introduced from India and Montserrat (Leeward island) during 1970-71, and from St. Vincent (Windward island) and Montserrat during 1977-80 (Alam 1982). The parasite is indigenous to Jamaica, and is well distributed in the cabbage growing areas. It has a density-independent relation to pest populations.

Mating behavior: In the laboratory, the newly emerged males and females were placed together in glass vials, fed on 10% honey solution and observed. The male mounts the female from behind to insert the aedeagus. Mating lasts from 15-20 seconds and multiple matings occur.

Mode of parasitism: Some 24 hours after mating, the parasites are provided with full-grown larvae of DBM in glass jars or glass tubes, in the ratio of three females to one host larva for 48 hours. The female usually mounts the caterpillar from its lateral side and gently inserts the ovipositor into the cuticle to deposit eggs subcutaneously. After the exposure period, the larvae were isolated in 7 × 2 cm glass vials and provided with pieces of cabbage leaves for food until pupation. In Jamaica at ambient temperature, the total development period (egg laying to adult emergence) lasts for 15-26 days. In Barbados the life-cycle was completed in 12-13 days (Alam 1982). The number of parasite adults raised from a single pupa ranged from 3 to 40. In the laboratory, 43.3-96.6% DBM larvae were successfully parasitized.

Levels of parasitism: The field parasitism ranged from 0 to 4.7% from April to December 1988, and 0 - 2.6% from February to December 1989 in Douglas Castle; 0 - 19.5%

during March, May, August and November 1988 and 0 - 15.9% from February to December 1989 in Castle Kelly; 0.5 - 12.8% from April to December 1989, and 2.4% in January and 7.8% in March 1990 at Bodles Agricultural Experimental Station; and 0 - 2.9% in Blue Mountains.

Following release in Barbados the parasite was recovered from many cabbage fields. The levels of parasitism recorded were 67.7 - 100% in 1976 and 26% in March and 12.5% in April 1980. Probably due to persistent use of chemical pesticides against DBM and other cruciferous pests, the parasite failed to maintain high levels of parasitism in the fields (Alam 1982). Forbes and Mansingh (1990) reported 0 - 7.1% parasitism in 1986 and 0 - 10.4% in 1987 in Jamaica.

Cotesia (= *Apanteles*) sp. (*glomeratus* group)

This indigenous larval parasite of DBM was recorded in all observation sites. The parasite prefers 2nd and 3rd instar larvae, but under laboratory conditions it also attacks full-grown caterpillars. Generally the field parasitism was erratic and very low. During 1988 the levels of parasitism recorded were 4.5% in May and 5% in August in Castle Kelly, 1.5% in June in Douglas Castle, and 3.7% in June in Blue Mountains. The parasite was not recorded during 1989 and 1990.

Trichospilus diatraeae Cherian and Margabandhu

This pupal parasite of lepidopterous insects is of Indian origin. It was recorded only once from a DBM pupa collected in Douglas Castle in April 1989.

In Barbados the parasite was introduced from India in 1972 (Alam and Gibbs 1984) against lepidopterous pests including *S. frugiperda* and was reared from field-collected pupae of CL, *Pseudoplusia includens* Walker, *Diaphania hyalinata* (L.) and *Stemorrhages flegia* (Cramer) (Bennett and Alam 1985). After the parasite was introduced into the other Caribbean islands (Alam 1978; Alam and Gibbs 1984), recoveries were made from the field-collected pupae of *D. hyalinata* and DBM in St. Vincent (Alam, 1986, CARDI's unpublished visit reports).

In 1984, M. Yaseen reared *T. diatraeae* from a noctuid pupa (possibly *S. frugiperda*) collected among rice stubbles at Caroni, Trinidad. On 3 May 1985, H. Glenn collected 48 pupae of *Epimecis detexta* (Walker), a geometrid pest of avocado from a commercial grove in Dade County, Florida, USA, and two of these yielded adults of *T. diatraeae* (Bennett et al. 1987).

Since Bennett et al. (1987) postulated that *T. diatraeae* was established in Trinidad as the result of escapes while the species was undergoing host-range tests, this should not account for its presence in Florida. Its appearance in North Florida (Gainesville) as well as in South Florida (Homestead) indicates that it is probably widespread in Florida. Whether it arrived there as an accidental immigrant direct from Asia or from Barbados cannot be ascertained. Similarly, the source of *T. diatraeae* in Jamaica is not clear. Probably it arrived here with the planting materials brought from the eastern Caribbean islands or carried by hurricane winds.

Predators

The most common groups of predators found in cabbage fields were coccinellids, chrysopids, syrphids and staphylinids (Table 1). Although their exact contribution in regulating the pest populations has not been evaluated, it is expected that these play an important role in the overall mortality of DBM and other cruciferous pests in the field. There is a need to conserve the populations to enhance their effectiveness, by reducing the indiscriminate use of pesticides.

Fungi

Three species of fungi (*Beauveria bassiana*, *Hirsutella* sp. and *Paecilomyces* sp.) were found infecting the larvae and pupae of DBM at Douglas Castle and Castle Kelly. The fungi were

Table 1. A complex of parasites, predators and fungi attacking various stages of DBM in Jamaica.

Name	Status
Parasites	
Hymenoptera	
Trichogrammatidae	
Trichogramma sp.	Egg-parasite
Ichneumonidae	
Diadegma insulare (Cresson)	Larval parasite
Braconidae:Cotesiini	
Cotesia (= Apanteles) sp. (glomeratus group)	Larval parasite
Pteromalidae	
Oomyzus (= *Tetrastichus*) *sokolowskii* (Kurdjumov)	Larval-pupal parasite
Eulophidae	
Trichospilus diatraeae Cherian and Margabandhu	Pupal parasite
Hyperparasites	
Ceraphronidae	
Aphanogmus (= *Ceraphron*) *fijiensis* (Ferriere)	Pupae of *Cotesia* species
Eulophidae	
Horismenus sp.	Pupae of *Cotesia* species
Pteromalidae	
Catolaccus sp. (species new to science)	Pupae of *Cotesia* species
Chalcididae	
Spilochalcis sp. (species new to science)	Pupae of *Diadegma insulare* and *Cotesia* spp.
Predators	
Coleoptera	
Coccinellidae	
Coleomegilla maculata (DeGeer)	Eggs and young larvae
Cycloneda sanguinea L.	Eggs and young larvae
Hippodamia convergens	Eggs and young larvae
Staphylinidae	
Belonuchus gagates Erichson	Prob. larvae and pupae
Diptera	
Syrphidae	
Toxomerus dispar (Fab.)	Young larvae
Toxomerus watsoni (Curran)	Young larvae
Pseudodoros clavatus (Fab.)	Young larvae
Neuroptera	
Chrysopidae	
Ceraeochrysa claveri Navas	Larvae
Fungi	
Beauveria bassiana	Larvae and pupae
Hirsutella sp.	Larvae
Paecilomyces sp.	Pupae

found mainly during the wet season, attacking some 5 - 10% of the larvae and pupae in the field. They were identified by Dr Chris Prior, Insect Pathologist, CAB-IIBC, Silwood Park, London. He cultured *B. bassiana* at Silwood Park and brought it to Jamaica for field trial.

Field Application: On 16 July 1990, the *Beauveria* formulation was sprayed in one cabbage field at Bodles. Observations on the larval mortality were carried out from 18 to 30 July 1990. Details are provided in Table 2.

The results showed that the field application of *B. bassiana* against DBM larvae caused high mortality, ranging from 12.5 to 54.5% in treated plots compared to 10.7 to 46.9% in the untreated plot. In the latter case the mortality was probably due to fungal spores drifting during the time of application, when the wind was fairly high. However this small experiment, using an indigenous pathogen against DBM has opened a new area for future research and development of this and other available pathogens (both indigenous and exotic) for the biological control of DBM and other cabbage pests.

Table 2. DBM Larval mortality caused by *Beauveria bassiana*, in sprayed and unsprayed plots, at Bodles Agricultural Experimental Station, Jamaica.

	Date larvae collected	No. larvae collected	No. larvae infected	Percentage infection
Sprayed plot	18 July 1990	69	30	43.5
	20 July 1990	112	14	12.5
	21 July 1990	112	61	54.5
	30 July 1990	112	47	41.9
Unsprayed plot	20 July 1990	56	6	10.7
	21 July 1990	160	75	46.9
	30 July 1990	112	39	34.8

Exotic parasites

Although a number of indigenous natural enemies attacking DBM have been reported from the eastern Caribbean islands, these have not provided sufficient control of the pest. It was, therefore, decided to introduce some well-known parasites from India, Pakistan and the eastern Caribbean islands to supplement the existing mortality levels of DBM. Alam (1974) reported the introduction of five parasite species into Barbados against DBM. Of those *Cotesia plutellae* (Kurdj.) from India, and *Oomyzus* (= *Tetrastichus*) *sokolowskii* (Kurdj.) from India, Montserrat and St. Vincent, became established (Alam 1982). *Cotesia plutellae* was successfully introduced into Barbados in 1969, and between 1971 and 1985 the annual average parasitism ranged from 17.9 to 52.5% (Alam 1982, 1986). It was also introduced into the eastern Caribbean islands with similar success (Alam 1986).

In Jamaica earlier introductions of *C. plutellae* and *O. sokolowskii* were not successful. They were again introduced from Barbados in March 1989 and soon after, *C. plutellae* was recovered from release sites at Mona Campus, University of the West Indies and Bodles Agricultural Experimental Station. At Bodles, from March 1989 to July 1990, the levels of parasitism ranged from 5.4 to 88.7%. The parasite is highly density-dependent. The level of parasitism increased with the increase of pest population and the converse was also true (Fig. 2). The parasite was also recovered from Douglas Castle, Castle Kelly and Blue Mountains, but the levels of parasitism were low. In the former locality it parasitized fewer than 1% larvae in 1989 and in Castle Kelly 2.8% larvae were parasitized in February 1989, while in Blue Mountain area it parasitized 3.8% larvae in June, 2.7% in July and 0.7% in September 1989. The reasons for failure of the parasite in Douglas Castle and Castle Kelly areas are not yet clear.

Hyperparasites

Two species of hyperparasites (*A. fijiensis* and *S. hirtifemora*) were found attacking the cocoons of *C. plutellae* in Barbados. Of these the former parasite was more abundant, attacking up to 13% of the cocoons during the rainy season. The hyperparasite *A. fijiensis* was also recorded from St. Vincent, attacking *C. plutellae* and other *Cotesia* spp. and *Apanteles* spp. (Alam 1982).

In St. Kitts A. *fijiensis*, S. *hirtifemora* and *Catolaccus* sp. were found attacking the cocoons of *C. plutellae*. Of these *S. hirtifemora* was the most abundant, attacking up to 50% parasite cocoons in the field. However, in spite of heavy damage to the *Cotesia* pupae, *C. plutellae* remained fairly abundant, parasitizing over 75% of the DBM population in the field (Alam 1980 to 1990, CARDI's unpublished visit reports).

After the establishment of *C. plutellae* at Bodles and at UWI Mona Campus in Jamaica, the parasite cocoons were attacked by four hyperparasites: *A. fijiensis*, *Catolaccus* sp., *Horismenus* sp. and *Spilochalcis* sp. The levels of parasitism by the former species ranged from 0 to 1.6%, *Horismenus* sp. 0 - 13.3% and *Catolaccus* sp. 0 - 6.3%. The more persistent hyperparasite was *Spilochalcis* sp. which was recorded throughout 1989 and 1990. The range of parasitism was 3.3 - 28.9%. However, the damage from these hyperparasites did not affect the efficiency of the primary parasite significantly.

Discussion

DBM is a cosmopolitan pest which thrives under extremely varied climatic conditions (Paramonov 1953). Although a temperature range of 17-25°C is considered optimum for the pest (Atwal 1955), DBM is the most serious pest of crucifers in the Polar regions of the USSR (Kutsenin 1977). Under tropical conditions, particularly in the Caribbean region, where the temperature fluctuates between 28-31°C throughout the year, the pest breeds continuously and completes its development in 14-24 days, suggesting up to 20 generations a year (Alam 1982).

In the Caribbean and particularly in Jamaica, highest populations of DBM were recorded during dry hot weather (January to June), and reduced to the minimum in the rainy season (July to December). Talekar et al. (1986) supported these observations in Taiwan, where during the dry period when the crop was irrigated with overhead sprinklers, the pest population was reduced significantly. Yaseen (1974) also reported that in Trinidad the pest populations were highest during periods of low rainfall.

A larval parasite *C. plutellae* and a larval-pupal parasite *O. sokolowskii* were successfully introduced into Barbados in the early 1970s, and the average annual larval parasitism by the former species ranged from 18 to 52.5% (Alam 1982 and 1986). *Cotesia plutellae* was later introduced into the eastern Caribbean islands where it became readily established, and along with *O. sokolowskii*, provided significant control of DBM. Soon after the release of *C. plutellae* in the fields in St. Kitts, it became established. The buildup of the parasite was phenomenal, where in spite of one to three applications of insecticide cocktails per week, an average 75% DBM larvae were parasitized. During field visits, it was generally observed that in unsprayed fields, a large number of *Cotesia* adults were hovering over cabbabge heads and many were actively searching for DBM larvae under cabbage leaves. In sprayed fields, although a number of parasite cocoons were found on cabbage leaves, the adults were virtually absent. In spite of repeated applications of different insecticides, many farmers failed to control the pest. When these fields were left unattended, the parasites moved in, built up large populations and spread to the newly planted fields nearby. The situation in Barbados and the other Caribbean islands is similar to that of St. Kitts, where the vegetable growers keep spraying their crops at regular intervals, with little or no control of DBM.

In Jamaica, where the complex of indigenous natural enemies is slightly different from that in the eastern Caribbean islands, the presence of *D. insulare* was considered an asset and the introduction of another larval parasite, particularly *C. plutellae*, could add to the larval mortality and reduce DBM populations. After the introduction of *C. plutellae* in April 1989, the parasite was released at Mona Campus, UWI, and Bodles Agricultural Experimental Station. It has shown some promise at Bodles, but in other areas although it has been recovered repeatedly, the populations remained low. Attempts to increase the parasite populations in all cabbage-growing areas by continuous releases of laboratory-reared adults are in progress.

The probable reason for the failure of *C. plutellae* to build up high populations, particularly in Castle Kelly, Douglas Castle and Blue Mountain areas, is the excessive use of pesticides and

the presence of a number of hyperparasites, viz. *Spilochalcis* sp., *A. fijiensis*, *Horismenus* sp. and *Catolaccus*. The former species is widely distributed in Jamaica, attacking large populations of *D. insulare* and now *C. plutellae*, whereas the other three species are more prevalent at Bodles and Mona, attacking mainly the cocoons of *C. plutellae*. In spite of these serious limiting factors, the primary parasite still maintains reasonably high populations in the fields providing significant control of DBM.

In St. Kitts, the cocoons of *C. plutellae* were attacked by another chalcid *Spilochalcis hirtifemora*, attacking up to 56% of the cocoons in the field. Occasionally small populations of *A. fijiensis* and *Catolaccus* sp. were also found attacking *Cotesia* cocoons. Although these hyperparasites limited the viability of *C. plutellae* populations, they did not prevent the rapid buildup of the primary parasite and its effect on DBM.

Our observations are almost similar to those of Sastrosiswojo and Sastrodihardjo (1986) in Indonesia, who reported that the establishment of *Diadegma semiclausum* in Indonesia provided up to 80% larval mortality in different parts of the country where DBM still remains the most important pest of crucifers.

Since the reintroduction of *C. plutellae* is very recent in Jamaica, the parasite has not yet been established in all the ecological zones of the country. It is hoped that the parasite will quickly get acclimatized to Jamaican conditions to increase its populations as in other Caribbean islands, and along with other indigenous natural enemies, provide better control of DBM. The addition of local fungi particularly *B. bassiana* and *Hirsutella* sp. may help achieve permanent control of DBM.

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Quantifying the Impact of Parasitoids on Diamondback Moth

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Abstract

The accurate measurement of parasitoid impact on diamondback moth, *Plutella xylostella* (L.) populations is important to both the evaluation of exotic parasitoids for introduction, and to the integration of parasitoids with other cropping practices, particularly pesticides. Parasitoid impact is usually measured by samples for percent parasitism. Problems with the accuracy of this approach, and its application in tropical conditions, are discussed. Two other approaches, the recruitment and graphical methods, are outlined, and some data on preliminary trials with these methods are presented.

Introduction

The use of natural enemies in the management of diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), can be viewed as a two-step process. Because DBM is exotic over much of its range, the first step is to establish effective and specific natural enemies from its area of origin. The second step is to maximize the contribution of these natural enemies by manipulating cropping practices, particularly pesticide use. At present, pesticide use may be excluding the key natural enemies of DBM in many areas.

The ultimate aim must be to maximize the contribution of natural enemies, which may be free to the farmer, and to minimize the use of pesticides thereby achieving a sustainable and cost-effective integrated pest management strategy.

In some cases, it is clear that introducing one or more parasitoids of DBM may eliminate the need for chemical pesticides altogether (e.g. Cape Verde Islands). In other cases, particularly in lowland tropical areas, there may be a continuing need for pesticide application on the basis of a spray threshold. This threshold should incorporate the potential of both the pest and the natural enemies in the crop system.

Activities of IIBC in Biocontrol of DBM

The International Institute of Biological Control (IIBC) has been involved for many years in the effort to find and distribute effective natural enemies of DBM. As early as 1928, IIBC scientists began exploration for parasitoids of DBM in Europe, and in 1936 made the first ever shipment of DBM parasitoids to New Zealand (Thomas and Ferguson 1989). Since then, from its stations around the tropical world, IIBC has carried out studies on the biology of DBM parasitoids and provided these species to countries in Asia, Africa and Latin America. Current work focuses on Central America and West Africa.

From its early exploratory work, IIBC is acutely aware that the range of parasitoids used in the biological control of DBM is only a fraction of those available. Most exploration in Europe has so far concentrated in cooler regions, whereas the principal problems with DBM today occur in the hot tropics. Therefore, we see a clear need for renewed exploration for tropically adapted strains of parasitoids in the suspected European region of origin of the pest.

In recent years, IIBC research has begun to focus as well on the second step in the biological control of DBM, its integration with chemical control. Our research has been directed at the development of laboratory bioassays for pesticide effects on parasitoids, and the selection of pesticide-resistant strains (Ke et al. 1991).

In this paper, however, we wish to discuss another current area of interest which we feel is crucial to both steps in biological control, namely the accurate and quantitative assessment of the impact of parasitoids on DBM in the field.

The need for an accurate assessment of parasitoid impact is obvious to any scientist working with IPM of DBM. Without it we cannot understand the contribution of introduced parasitoids, we cannot determine which parasitoids species are more effective, and we cannot calculate how pesticides or other cropping practices affect their contribution.

Measurements Based on Percent Parasitism of Hosts

In principle, the impact of parasitoids on DBM can be measured in a number of ways. Lim et al. (1986), for instance, have used the insecticide check method to remove parasitoids, and other exclusion methods are possible (Luck et al. 1988). But in most instances, parasitoid impact has been measured by percent parasitism. The accuracy of this method depends on the way in which samples are made. A popular method, the comparison of parasitoid cocoons to healthy larvae, for instance, has so many sources of error that an accurate assessment is very unlikely. Such measures may, however, be of qualitative value, for instance, to compare two extreme treatments in nearby plots.

A more precise measure of percent parasitism comes from sampling host larvae and rearing (or dissecting) parasitoids from them. Even with this simple method, problems can arise. Three problems are particularly common.

Firstly, percent parasitism may be measured with no reference to the density of the host population on which it is acting. Such a measurement is of little practical use: there is a very substantial difference between 20% parasitism of a population of 1 insect/plant, and 20% parasitism of a population of 20/plant.

Secondly, the incorrect host stage is sometimes used for calculating percent parasitism, that is, the denominator contains hosts too young or too old to be attacked. Only stages attacked by the parasitoid should be sampled. Further, the greatest accuracy is obtained by sampling a host stage between that which is attacked by the parasitoid and that from which the parasitoid emerges (Van Driesche 1983; Van Driesche et al. 1991). If we sample earlier stages, we may be removing hosts from the field before they have been fully exposed to parasitism, hence we may underestimate parasitism.

Finally, parasitoids affect the development of their hosts, and this can lead to biases. Thus, a developing parasitoid may keep a host in one stage long after healthy hosts have moved to a later stage which is not sampled. This will lead to an overestimation of parasitism.

Some of these problems arise with parasitoids of DBM. *Diadegma semiclausum* Hellen will parasitize all larval instars, although it prefers the second and third (Lloyd 1940; Velasco 1982). Fourth instar larvae or prepupae may be the best stage to sample, but sampling fourths may remove them from some parasitism which would have occurred later. Further, host larvae parasitized in later instars spend longer in the fourth and prepupal stage (before producing a parasitoid) than healthy larvae (before producing a moth pupa) (Mallya 1980). Samples of prepupae, therefore, might overestimate parasitism.

For pupal parasitoids, like *Diadromus collaris* Gravenhorst and egg parasitoids, like *Trichogramma* spp., parasitized hosts remain as pupae and eggs for some period after healthy hosts of the same age have become adult moths or larvae, respectively.

For *Cotesia plutellae* Kurdjumov, samples of early fourth instars will give the most accurate estimate, as parasitism is concentrated on the second and third instar and emergence is from the fourth. However this parasitoid does affect the rate of host development, such that errors are possible (P.A.C. Ooi, IIBC, pers. comm.). There is little published information on the effects of *Tetrastichus sokolowskii* Kurdjumov on host development, but it has been observed that parasitism actually accelerates host cocoon formation (Cherian and Basheer 1939) and delays relative to healthy hosts. Both effects would tend to overestimate parasitism from pupal collections.

Problems with Measuring Parasitism by Host Samples

Let us assume that our measure of percent parasitism from samples of DBM is the most appropriate one to avoid the possible errors just mentioned.How do we use samples to get a meaningful measure of the impact of a parasitoid on this pest? The interval over which we commonly attempt to measure percent parasitism is the host generation. This generational mortality is a kind of common currency in studies of biological control. A measure of generational mortality tells us that parasitoid X reduced the probability of pest Y to survive and reproduce by a certain percentage. This measure can be compared between different pest densities, cropping practices or sites, to determine what factors promote or limit biological control.

By contrast, one or a few samples of pests from which parasitism is estimated represent little more than snapshots of this process and, depending on when they are taken and the local phenology of pests and parasitoids, they may vary greatly in its estimate of generational mortality.

Sometimes, however, a few samples of percent parasitism *can* give us an accurate measure of generational mortality from a parasitoid. This occurs when DBM has discrete generations and reasonably discrete life stages within these, such that a sample of the appropriate stage is really a sample of an entire single generation as it passes through that stage. In temperate regions, such distinct generations do occur, and have permitted Harcourt (1963), for instance, to estimate generational parasitism by *Diadegma insulare* (Cresson) in Canada by sampling the appropriate DBM life stages in a single generation.

Alternatively, if DBM has completely overlapping generations, and mortalities due to parasitoids and other factors are not changing over time, then any sample of the appropriate stage at any time will give a good measure of generational parasitism. This method is described in Southwood (1978).

Unfortunately, the population biology of DBM in many tropical countries usually falls between these two extremes. Typically, when a brassica crop is transplanted to the field, adult moths lay eggs and produce an initial generation, which may be fairly discrete. But as the population grows, generations begin to overlap, until at the time of harvest, perhaps six generations later, all life stages are present at all times.

A typical pattern for a DBM population in a cabbage field in Honduras is illustrated in Fig. 1. These data come from the fourth cabbage crop on a field where cabbage was cropped continuously, in an overlapped manner, as is common in Honduras and other tropical regions. In this situation, it is clear that waves of young larvae are entering the population, but it is not possible to identify clearly a single generation.

In Fig. 2, percent parasitism of fourth instar larvae and prepupae by *D. insulare* is shown from the quantitative samples of larvae and prepupae shown in Fig. 1. These samples were held in the laboratory for moth or parasitoid emergence. Like the host populations, parasitism typically shows dramatic fluctuations over time, between 0 and about 50%.

Most data on parasitism of tropical DBM populations are presented in the form shown in Fig. 2. It gives a qualitative impression of the relationship between parasitism and pest density,

which may be compared with patterns from other times, sites and treatments. But it does not permit a quantitative measure of mortality such as generational parasitism, because discrete generations cannot be distinguished.

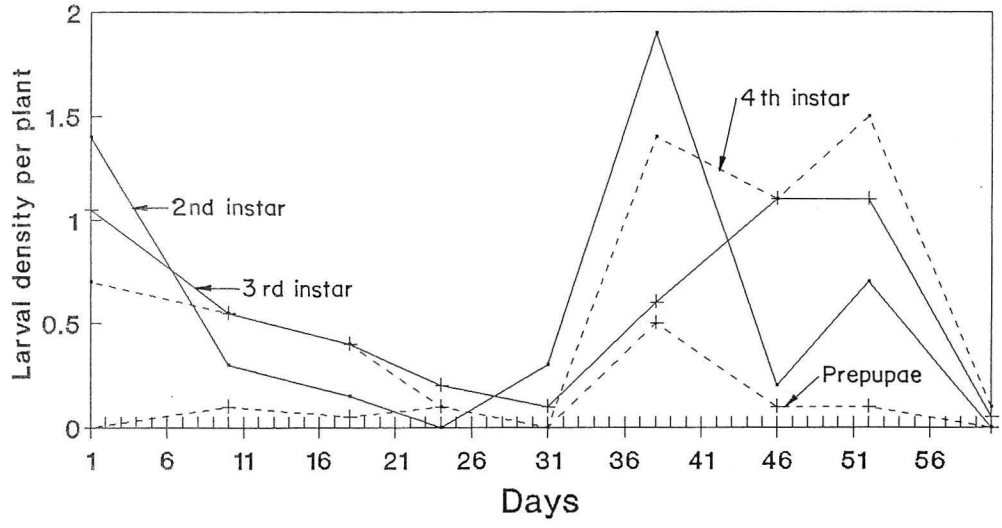

Fig. 1. An example of age structure in a tropical DBM population, which is clearly intermediate between discrete and completely overlapped generations. Data from a cabbage field in Honduras, Aug-Oct 1989 (A.J. Cherry, unpublished data).

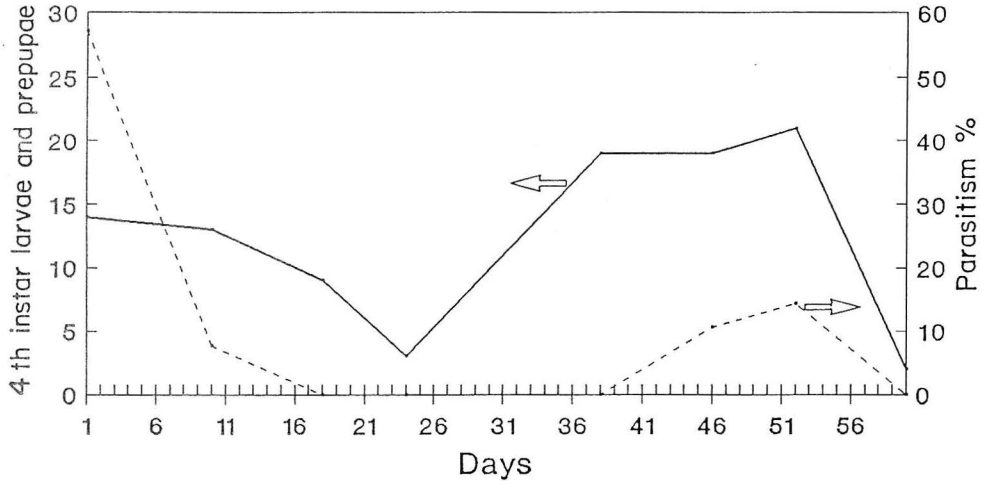

Fig. 2. An example of DBM populations and levels of parasitism by *D. insulare*, from same samples as for Figure 1, to illustrate typical fluctuations (see text).

A Possible Approach to Quantifying Parasitoid Impact

Measuring the impact of parasitoids on DBM poses two problems: measures of percent parasitism are subject to error, and tropical populations of DBM rarely have sufficiently discrete generations such that measurements of percent parasitism can estimate generational parasitism.

A possible solution to these problems comes from the recent exploration of alternative methods to estimate generational mortality due to parasitism (Van Driesche et al. in press). Two

of these methods, which we shall call the 'recruitment' and 'graphical' methods, involve sampling the number of susceptible hosts in a population over time and, later, the number of parasitoids actually produced from these. The recruitment method (Van Driesche and Bellows 1988) measures the rate at which hosts entered susceptible stages and were parasitized, while the much older graphical method (Southwood and Jepson 1962) estimates the total density of susceptible larvae and parasitoids produced over the selected interval.

These methods measure mortality over a selected interval, and are not constrained to a single generation. Since identifying discrete generations is difficult for tropical DBM populations, we suggest that they, and other methods for estimating impact, are best calculated over the interval of the brassica crop. In tropical brassica production, the crop period identifies a discrete unit of pest population growth, plant damage and crop yield. The damage caused to a tropical crop by five to ten successive DBM generations between transplanting and harvest is, to some extent, cumulative in its effect on yield. Similarly, parasitoid action has a cumulative effect on the growth of pest populations, and hence on damage and yield. For this reason, the crop period seems an appropriate interval over which to quantify the contribution of parasitoids to DBM management.

Below we report some preliminary efforts to apply the recruitment and graphical methods to the measurement of parasitism of DBM over a crop period. Studies were made in Honduras on cabbage fields in collaboration with staff of the Escuela Agricola Panamericana, El Zamorano. We report results from one site and crop, by means of illustrating the two methods. An expanded, more detailed account will be published elsewhere by the junior author (A. Cherry, IIBC, unpublished data).

Recruitment method. This method has been described by van Driesche and Bellows (1988) for another pest of brassicas, *Pieris brassicae*. It estimates the rate at which host larvae recruited into the stage susceptible to parasitism (i.e. from an earlier stage) over a particular period, and the rate at which parasitoids are recruited into the adult parasitoid population from these larvae at a later time. Over an interval, the ratio of these rates gives a measure of the proportion of susceptible larvae in the population which ultimately died from parasitism.

A single cabbage crop was sampled from transplanting to harvest at intervals of 3-7 (average 4.3) days. On each sampling date, 10 plants were chosen at random from the crop and marked with stakes. With the exception of first instars, all larvae, prepupae, pupae and parasitoid pupae were removed from each plant. Second and third instar larvae were discarded, and fourth larval and prepupal stages retained on fresh leaves in the laboratory at ambient conditions of temperature and light. After 48 hours the same 20 plants were inspected and a larval count made. During the period between sampling and inspection, eggs and first instar larvae left on the plant had developed into the susceptible stage. Thus, assuming that there was no immigration from other plants and that the plant was perfectly cleared during sampling, all larvae present at inspection represented recruitment to the susceptible host population during the given period. A plant once sampled was not selected again. Figures were adjusted to give an estimate of recruitment over a 24-hour period.

Recruitment to the *D. insulare* parasitized host population was estimated from the original sample of fourth instar larvae and prepupae. As with host recruitment, samples were recorded for parasitoid cocoon production over 48 hours, and estimates derived for recruitment over 24 hours. The possibility that parasitized hosts developed more slowly, and hence were over-represented in samples of fourth instar larvae and prepupae (as mentioned above for *D. semiclausum*), is a source of error which would overestimate parasitism.

This method for measuring parasitoid recruitment varies from that of van Driesche and Bellows (1988) who measured recruitment at the parasitoid egg stage, by dissection of field-collected larvae. Our method incorporates the possible error that, between parasitism and parasitoid emergence, parasitized hosts suffer different mortality than unparasitized ones. Further, during this period, parasitized hosts will die from other causes, such that our measure is more one of 'irreplaceable mortality' (Southwood 1978) due to parasitism.

The rates of recruitment arising from this method are plotted as daily rates relative to sampling dates (Fig. 3). The points are joined up and the area under the curves estimates the total recruitment of hosts and parasitoids.

Graphical method. In this method, the numbers of larvae of each stage sampled on each date are plotted on a graph (Fig. 4). At the end of the sampling period, the points are joined

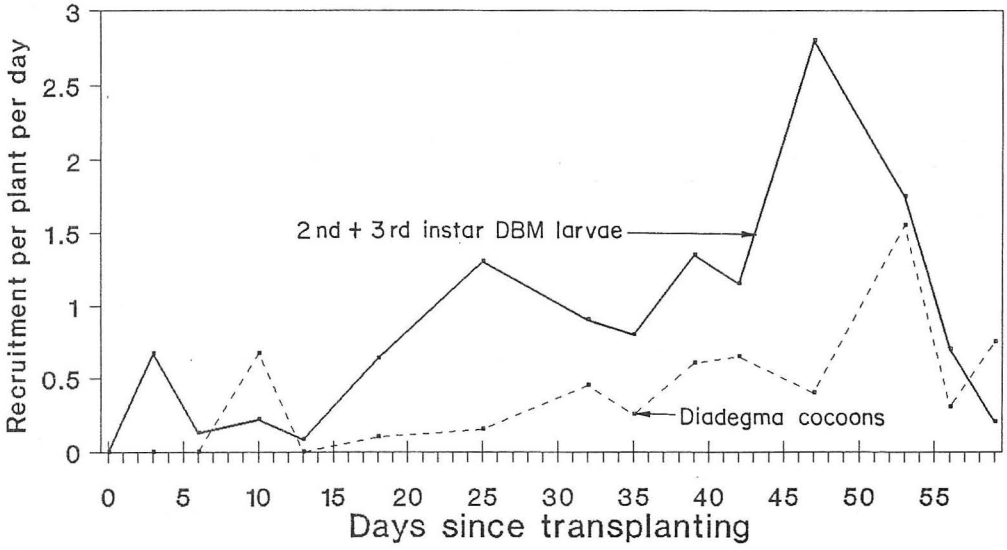

Fig. 3. Recruitment curves for Instars II and III of DBM and *D. insulare* pupae over a single cabbage crop in Honduras, 14 Dec-13 Feb 1989 (A.J. Cherry, unpublished data). See text for explanation.

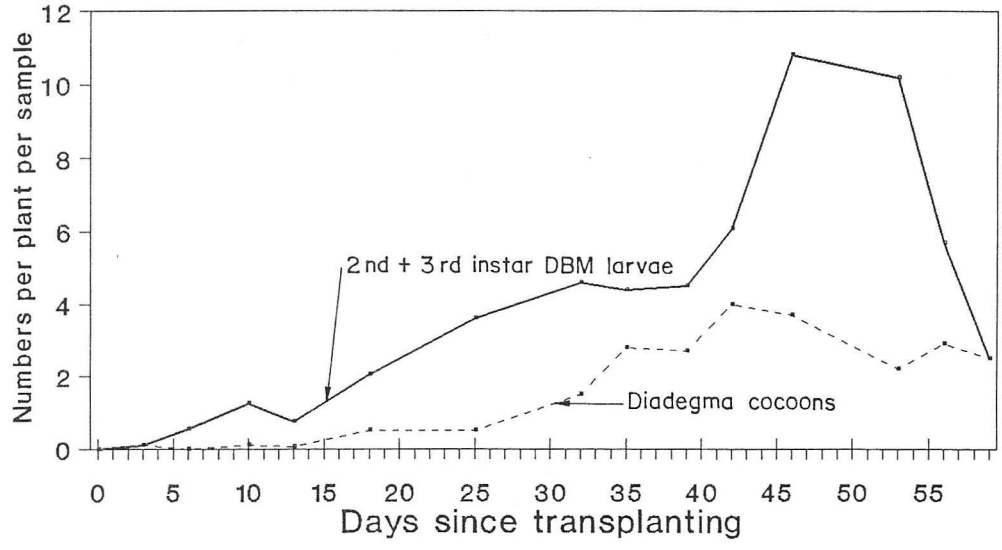

Fig. 4. Sampling results for Instars II and III of DBM and pupae of *D. insulare* over the same crop as for Figure 3 (A. J. Cherry, unpublished data). The area under these curves, divided by the development period for the particular stage, yields and estimate of the total number of hosts and parasitoids produced during that crop, for use in the graphical method. See text for further explanation.

up and the area under the line is determined. This total is then divided by the mean development time under field conditions to give an estimate of the total numbers in that stage during the interval. Development times for the second-third instar period were estimated at 5.5 days, based on laboratory and literature data and temperature measurements in the field.

In this way, the total number of susceptible larvae in the second and third instar during the cropping period was calculated. Total numbers of parasitoids produced in the crop period were calculated in a similar manner by plotting the density of intact (e.g. unemerged) parasitoid cocoons at each sampling date, measuring the area under this curve, and dividing by the mean development time for parasitoid pupae (estimated at 6.5 days; Carballo and Quezada 1987).

As with the recruitment method, this method is subject to errors due to differential mortality of parasitized and healthy hosts between parasitism and parasitoid emergence, and it does not count parasitism of hosts which die of some other cause in the interim. The graphical method also makes other assumptions which weaken its accuracy, and is sensitive to errors in development times and effects of parasitoids on host development (Bellows et al. 1989; Groden et al. 1990).

Results and Discussion

The recruitment method is taken in this study as giving the most accurate measure of real levels of mortality due to *D. insulare* over the cropping period, despite the sources of error identified above. It produced an estimate of parasitoid-induced mortality over the crop period of 44.4%. The same estimate from the graphical method produced was 30.5%. Without replication, i.e., over consecutive crops or different treatments, the results of these two methods cannot be properly compared, but their similarity in this preliminary trial is encouraging. By contrast, independent estimates of percent parasitism from fourth instars and prepupae sampled on particular dates during the same crop ranged from 7 to 72% parasitism (A. Cherry, IIBC, unpublished data).

For scientists familiar with the sampling of hosts and scoring of parasitism by rearing or dissection, the recruitment and graphical methods may appear rather complex. The recruitment method, in particular, requires that measurements of recruitment be made in the field, as well as estimation of parasitism rates from collected samples.

By contrast, the graphical method appears very simple requiring only population samples which might be made in the course of a study of the pest population alone. This desirable simplicity, however, is offset by biases created when its assumptions are not met (Bellows et al. 1989; Groden et al. 1990).

Conclusions

This paper has sought only to identify problems with estimating parasitoid impact on DBM and to suggest some possible solutions. Considerably more work is necessary to test quantitative methods for assessing impact, in order to determine which methods best combine accuracy with ease of implementation. In the course of this, other approaches should be tried as well, such as the so-called 'death rate analysis' (Van Driesche et al. 1990), which has only recently been developed.

Although we have identified problems with present methods for measuring percent parasitism and representing it as seasonal trends, we do not urge abandonment of this approach at this time. It does provide information on trends in parasitism, qualitative information for comparing sites and treatments and, at present, it has few proven alternatives.

Nonetheless, the need to develop better quantification can be argued in the context of possible future trends in DBM management. We identify four such trends. First, we suggest that there will be an increased interest in the comparison of different parasitoid species and strains in combinations, particularly in lowland tropical areas.

Secondly, there will be an increasing use of selective pesticides (e.g. IGRs and microbials). It is easy to think that such a trend will mean that we need not worry about pesticide effects on parasitoids, but this is not true. Whatever pesticides we use against DBM, we invariably kill parasitoids, either as larvae inside dying hosts, or as adults, or both. Further, parasitoids are not only affected by direct lethal effects, but by sublethal effects and by 'indirect' effects, such as the effect of pesticides on lowering pest density, which may influence the searching efficiency of the parasitoids (Waage 1989). From a practical viewpoint, we will need methods to compare different selective products which may differ more subtly in their effect on parasitoids than has been apparent in the broad-spectrum vs selective pesticide comparisons of recent years (e.g. Lim et al. 1986).

Thirdly, we will need to incorporate parasitoid action into decisions for spraying. This will require a means to quantify parasitoid impact over a short time-scale.

Finally, we will need in future to manage pesticide resistance, even to microbials. While it is not yet clear how this will best be done, rotation of pesticide use is one option. Biological control is itself a powerful tool for pesticide resistance management, because it can reduce the need for application and hence the rate of development of resistance to a new pesticide.

Managing pesticide use in the long term will also mean we need to manage biological control over similar periods, beyond the short-term decisions of whether or not to spray. As we change pesticide use, perhaps shifting from one to another, for resistance management, we will require a quantitative measure to monitor changes in natural enemy impact as well.

For all these future trends, threshold spraying and resistance management, a more quantitative approach to measuring the impact of parasitoids and other natural enemies will not only be desirable from a scientific standpoint, but necessary from a practical standpoint if we are to incorporate biological control effectively in IPM systems.

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Role of Parasitoids in Managing Diamondback Moth in the Cameron Highlands, Malaysia

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Abstract

Three major primary parasitoids of the diamondback moth, *Plutella xylostella* (L.), are found in the Cameron Highlands, Malaysia. *Cotesia plutellae* (Kurdjumov) was discovered in the early 1970s, *Diadegma semiclausum* (Hellén) and *Diadromus collaris* (Gravenhorst) were introduced in the mid 1970s from New Zealand and Australia. Field studies in the early 1980s showed that *C. plutellae* was the dominant parasitoid. This was contrary to laboratory studies which showed that *D. semiclausum* was an intrinsically superior parasitoid. In the Cameron Highlands, farmers sprayed insecticides frequently, often at concentrations far exceeding recommended dosages. In 1987, Singapore imposed restrictions on excessive levels of pesticide residues in crucifers. This together with high levels of insecticide resistance in the DBM resulted in farmers switching to the use of *Bacillus thuringiensis*, resulting in an unprecedented development for the management of diamondback moth. Even with reduced usage of chemical insecticides farmers were able to harvest good crops. Reduction in use of chemical insecticides allowed the primary parasitoids to realize their potential. The impact of biological control is manifested in: a) lower population of DBM despite less usage of insecticides; b) *D. semiclausum* became the dominant parasitoid; c) farmers realized that they need not be dependent on insecticides. Hence after more than 10 years, the impact of *D. semiclausum*, which was masked by excessive insecticide usage, was realized. Other parasitoids, predators and microbial agents probably act in consonance to suppress the DBM population. The experience in the Cameron Highlands emphasized the central role of parasitoids in managing diamondback moth. The strategy in managing this pest is to build up a core of effective parasitoids and supplement the action of parasitoids with use of *B. thuringiensis* when necessary.

Introduction

It was evident from reports of insecticide resistance in diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) (Henderson 1957; Lim 1972; Ooi and Sudderuddin 1978; Sudderudin and Kok 1978), that the approach adopted by cabbage farmers in the Cameron Highlands had to change. As the pest is an exotic insect, a likely approach was to import natural enemies of the DBM from its native home. This approach was attempted in New Zealand (Muggeridge 1930; Hardy 1938) and resulted in significant decline in DBM populations there (Todd 1959). Similarly, parasitoids of DBM were introduced into Australia and contributed to a useful level of control (Wilson 1960). Nearer home, an ichneumonid was introduced from New Zealand to Indonesia in 1950 and was an important parasitoid of DBM (Vos 1953; Sastrosiswojo and Sastrodihardjo 1986). These records suggested that biological control of DBM is feasible.

With that in mind, a biological control program for DBM was initiated in 1975. This paper reviews the status of the biological control program for DBM in Malaysia with a view to establishing a strategy for the role of parasitoids in the overall management of DBM.

Materials and Methods

To facilitate a review of the biological control of DBM, field data from ecological studies carried out in 1976-78 (prior to release of the major parasitoids) (Ooi 1979a) were compared with data collected from 1988-90 from a similar site in the Cameron Highlands. The site was the MARDI Research Station at Tanah Rata. In the studies, cabbage was grown in overlapping crops to ensure continuous sampling. No insecticide was used in the study. Twenty to thirty cabbages were removed every fortnight to examine for DBM and major parasitoids. Data from both studies were graphed to show population trends of DBM (larvae and pupae) and the number of cocoons of the major parasitoids observed. The population data were also analyzed using the method of Kuno and Dyck (1985), where a cabbage crop was divided into four crop periods. Each period corresponded to 27 days, being the average length of the DBM life cycle in the Cameron Highlands (Ho 1965). This analysis will indicate seasonal changes in the DBM population and allow comparison between two different sets of data. A similar analysis was reported in Ooi et al. (1990) for smaller but similar sets of data. Period I lasts from day 4 to 30, period II from 31 to 57, period III from 58 to 84 and period IV lasts from day 85 to harvest. Data from nine crops grown continuously between 1976 and 1978 were grouped into the respective crop periods, transformed using log (x+1) and averaged. Data from 11 continuous crops grown between 1988 and 1990 were similarly treated. The results were plotted to show the population trend. The regression between number of parasitoid cocoons collected and the respective DBM population was determined using a Lotus spreadsheet.

Biological Control of DBM in Malaysia

Before the 1970s, little was known of the ecology of DBM in Malaysia. The only control measure available to farmers was application of insecticides and this unilateral approach encouraged development of extensive insecticide resistance in the DBM (Ooi 1986; Ooi and Sudderuddin 1978; Sudderuddin and Kok 1978). Use of chemicals contributed to 30% or more of the total cost of production (Lim 1972). A biological control approach was simultaneously initiated by the Malaysian Agricultural Research and Development Institute (MARDI) and the Crop Protection Branch of the Department of Agriculture.

Studies by Lim and Ko (1975) resulted in the discovery of *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae) in the Cameron Highlands. It is unlikely that *C. plutellae* is native and could have arrived fortuitously towards the late 1960s. Levels of parasitism averaged 29.6 to 35.8% (Lim 1982) and in another study ranged between 12.3 and 19.1% (Ooi 1979a). The level of parasitism suggested that *C. plutellae* was not a very effective parasitoid. This contrasted with recent studies from Sabah where parasitism averaged 59.3 and 66.6% from two study sites (Tay et al. in preparation). *C. plutellae* was introduced into Sabah from India between 1971 and 1974. Initially, this parasitoid did not have any impact on the DBM population in the Kundasang Highlands and was thought to have failed to establish. However, studies in 1987 showed that this parasitoid was very common and appeared to keep the DBM in check in cabbage farms at Kundasang.

The levels of parasitism by *C. plutellae* in the Cameron Highlands appeared to be similar to that recorded in India (Bhalla and Dubey 1986; Joshi and Sharma 1974), Japan (Uematsu et al. 1987), Philippines (Velasco 1983) and Taiwan (Fan and Ho 1971).

Despite the low incidence of *C. plutellae* in Cameron Highlands, Lim (1986) suggested that this parasitoid is important in the development of an integrated pest management program (IPM) for DBM. This suggestion was tested by Lim et al. (1986) in an insecticide-check experiment. From the results obtained, it could be extrapolated that an IPM program would be better if there were more species of parasitoids acting on DBM at the different immature stages.

Four species of parasitoids were introduced into the Cameron Highlands from India, New Zealand, Australia and Indonesia (Ooi and Lim 1989). Of these, only two established, namely, *Diadegma semiclausum* Hellén (Hymenoptera: Ichneumonidae) and *Diadromus collaris* (Gravenhorst) (Hymenoptera: Ichneumonidae). The third ichneumonid *Macromalon orientale*

Kerrich (Hymenoptera: Ichneumonidae)was not released as it failed to breed in the laboratory. The fourth imported parasitoid *Tetrastichus sokolowskii* Kurdjumov (Hymenoptera: Eulophidae) was introduced into the Cameron Highlands and Kundasang but in both places failed to establish.

Like *C. plutellae*, *D. semiclausum*, *M. orientale* and *T. sokolowskii* attacked DBM larvae. *D. collaris* is a pupal parasitoid which attacks fresh DBM pupae within their cocoons. Details of the biology of *D. eucerophaga* and *T. sokolowskii* under Malaysian conditions are provided by Ooi (1980, 1988).

Status of parasitoids of DBM

Following the release of three parasitoids in the Cameron Highlands (Ooi and Lim 1989), only *D. semiclausum* and *D. collaris* established. For more than a decade, the impact of both parasitoids was not realized and in the minds of many, the introduction was unsuccessful. In 1987, complaints and rejections of vegetables with high levels of pesticide residues from an importing country encouraged farmers to reduce the usage of insecticides. By 1989, cabbage farmers in most of the Cameron Highlands noted with satisfaction that even with less usage of insecticides, the DBM problem was not serious. A study of the data from 1977 and 1989 showed that the DBM population in 1989 was reduced by about eight times or more as compared with that in 1977 (Fig. 1 and 2). Although the data were from an ecological study site, they

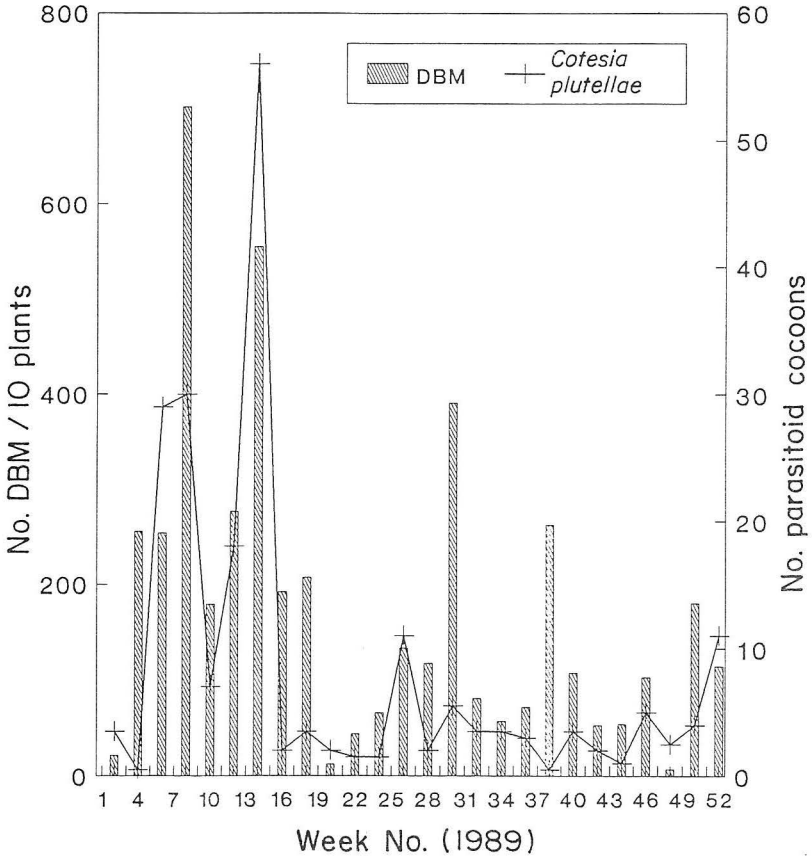

Fig. 1. Populations of DBM and *C. plutellae* (cocoons only) sampled at the MARDI Research Station, Tanah Rata in 1977.

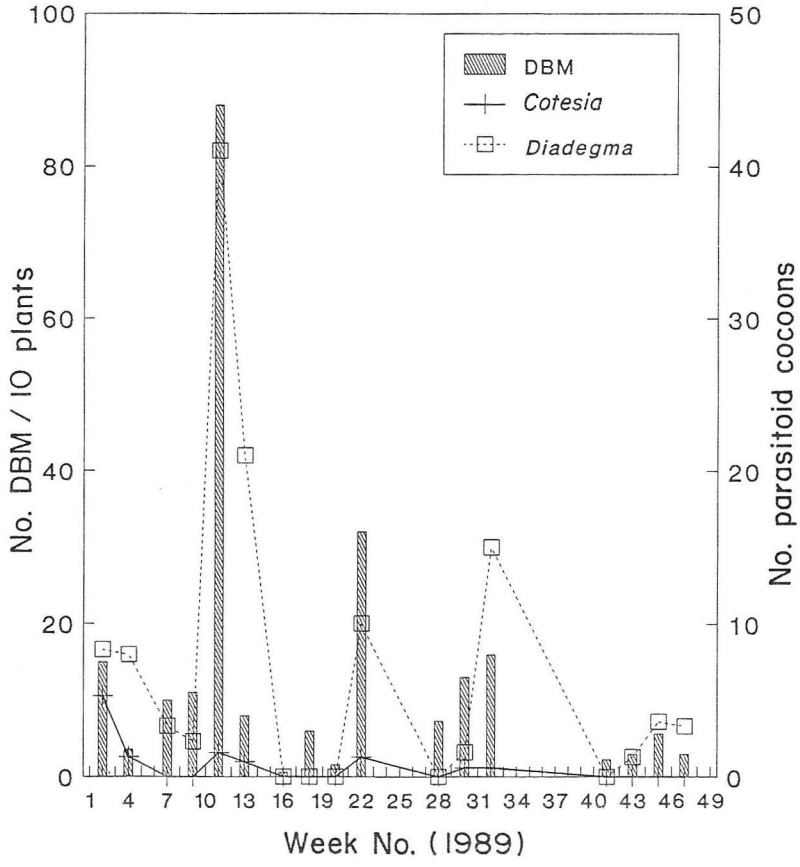

Fig. 2. Populations of DBM and two major parasitoids (cocoons only) sampled at the MARDI Research Station, Tanah Rata in 1989.

suggested that the overall DBM problem was very much reduced following the change in pest management practices. Farmers reported the use of *Bacillus thuringiensis* Berliner to replace chemical insecticides, and this had fewer adverse effects on the parasitoids, particularly the pupal parasitoid.

The differences in populations of DBM became more apparent when the seasonal changes for both periods were compared (Fig. 3). Besides being lower for 1988-90, the graph suggested that the DBM population did not grow as fast as in 1976-78 and also declined sharply after crop period II. A very effective mortality factor is suspected to act on the DBM population in the period 1988-90. As the crop was free of insecticides, the only explanation was the impact of parasitoids.

In 1976-78, the key parasitoid was *C. plutellae* and as noted in Fig. 1 did not appear to suppress the pest population. In the data of 1988-90, the dominant parasitoid was *D. semiclausum* and *C. plutellae* actually became rather rare (Fig. 2). This changeover supported the laboratory studies of both parasitoids reported by Chua and Ooi (1986) and Ooi (1980). *D. semiclausum* was superior in terms of its area of discovery and killing power (Table 1). In 1984, the dominant parasitoid was still *C. plutellae* and Chua and Ooi (1986) were puzzled by the field results. However, following the reduction in use of chemical insecticides, *D. semiclausum* exerted its dominance (Fig. 2). This dominance will continue as long as farmers refrain from unnecessary use of chemical insecticides.

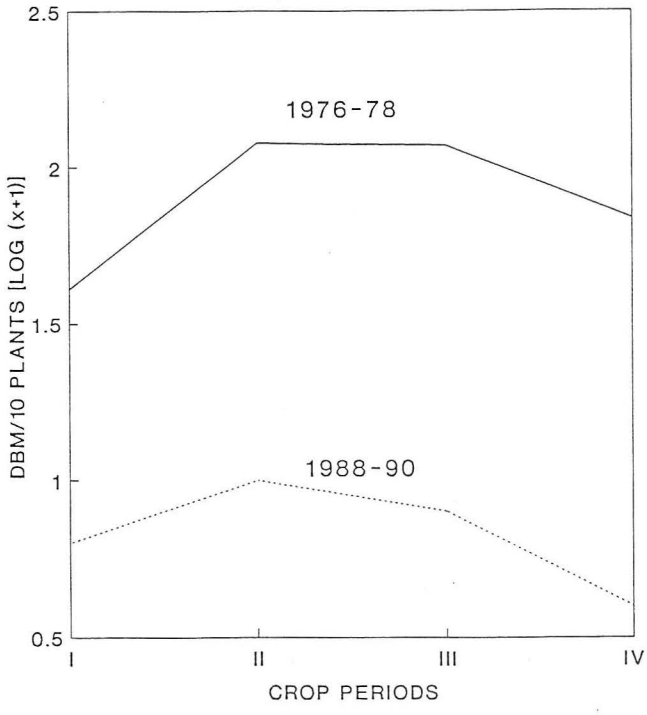

Fig. 3.
Seasonal population changes of DBM for 1976-78 (prerelease) compared with that for 1988-90. The results indicate a conspicuous decline in DBM population 11 years after introduction and release of *D. eucerophaga*.

Table 1. Biological/ecological attributes of two major DBM parasitoids in Malaysia (modified from Chua and Ooi 1986).

Species	Life cycle (days)	Host stage attacked	a	K	Field parasitism		
					Country	Incidence (%)	References
Cotesia plutellae	11-14	larval	0.18	0.09	India	31	Bhalla and Dubey (1986)
					India	36.6	Joshi and Sharma (1974)
					Japan	20-60	Uematsu et al. (1987)
					Malaysia	29.6-35.8	Lim (1982)
					Malaysia	12.3-19.1	Ooi (1979a)
					Philippines	1.9-16.4	Velasco (1983)
					Taiwan	19.6	Fan and Ho (1971)
Diadegma semiclausum	12-19	larval	0.38	0.87	Australia	29	Yarrow (1970)
					Indonesia	5.7-88.9	Sastrosiswojo and Sastrodihardjo (1986)

a = area of discovery (searching efficiency). K = Killing power.

A study of the relationship between *C. plutellae* and *D. semiclausum* and the DBM population suggested that both parasitoids were numerically responsive to increasing populations of DBM. Data from 1976 to 1978 showed that the relationship was represented by the linear graph $Y = 0.07X - 0.29$ ($R^2 = 0.54$; df = 41) for *C. plutellae*. In the data set of 1988-90, the relationship was represented by $Y = 0.06X + 0.11$ ($R^2 = 0.21$; df = 43). For *D. semiclausum*, the relationship with DBM was represented by $Y = 0.33X + 0.12$ ($R^2 = 0.41$; df = 43). Although *C. plutellae* was numerically responsive to increasing DBM populations, its *a* and K values (Table 1) suggested that it could not keep the pest population down. This is supported by results from studies in other countries which showed that levels of parasitism rarely exceeded 60%. Results from Sabah suggested that further detailed studies (including taxonomy of the insect) are necessary in Sabah to understand the impact of this braconid. *D.*

semiclausum possessed a significant positive numerical response and has good searching and killing power which would explain why it became the dominant parasitoid. The impressive impact of *D. semiclausum* on DBM was also reported by Sastrosiswojo and Sastrodihardjo (1986) in Indonesia and by Talekar in Taiwan (pers. comm.).

Biological Control as Core in DBM Management

The outstanding impact of parasitoids in the management of DBM in the Cameron Highlands has confirmed Lim's (1986) views. The need for key parasitoids should feature strongly in all integrated pest management programs. With a biological control core, it is then possible to integrate with other methods of control including judicious use of insecticides. However, experience in the Cameron Highlands suggests that *B. thuringiensis* should be preferred over chemical insecticides.

More research is necessary to prove if a single key parasitoid is sufficient to manage the DBM. In the Cameron Highlands, a complex of natural enemies is now linked to DBM (Fig. 4). The two major parasitoids, *D. semiclausum* and *C. plutellae*, attack the larval stages of the DBM. Should the larvae escape from the larval parasitoids, there are two pupal parasitoids that may act on the pupae. It is very likely that all the parasitoids work together towards achieving the level of natural biological control observed and no one parasitoid could sustain this impact. Conservation of parasitoids will invariably conserve predators and perhaps sustain the effect of microbial control. Little is known of the impact of predators, and there should be further study into how this mortality factor operates.

While biological control of DBM exists in the highlands, management of the DBM in warmer areas should receive further attention. Following the above strategy, the first step is to develop a complex of parasitoids and other natural enemies of DBM. As such, a program to explore for parasitoids in the warmer parts of its native range (e.g. Mediterranean) should be initiated.

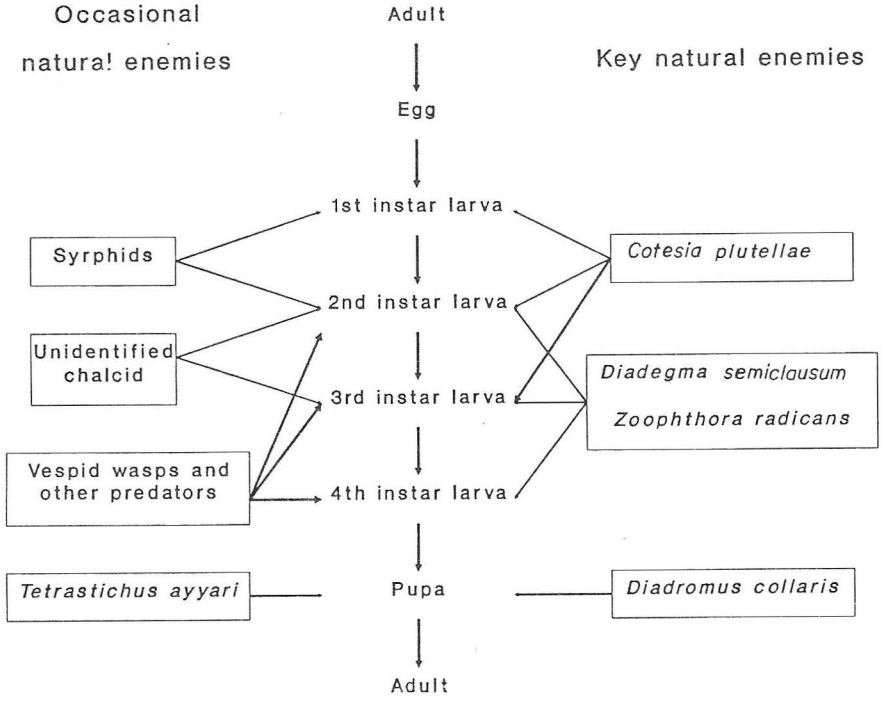

Fig. 4. Diagrammatic representation of linkages between DBM and its natural enemies in Malaysia (adapted from Lim 1982; Ooi 1979b; Ooi et al. 1990).

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Introduction of *Diadegma semiclausum* to control diamondback moth in Taiwan

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Abstract

Diamondback moth, *Plutella xylostella* (L.), is the most destructive pest of cruciferous vegetables in Taiwan. As elsewhere in Asia, this insect in Taiwan has developed resistance to all chemical insecticides used for its control. Failing to find any suitable and sustainable control measure, *Diadegma semiclausum* Hellen – a larval parasite – was introduced to combat diamondback moth in Taiwan. Despite repeated releases, this parasite did not establish in lowlands probably because of higher temperatures coupled with indiscriminate use of chemical insecticides by the farmers. However, a single release in the highlands resulted in the establishment of this parasite, probably because of cooler temperatures and relatively less intensive use of chemicals. This has resulted in reduction in the population of diamondback moth in the highlands. In laboratory studies, *D. semiclausum* parasitism was high at 15 to 25°C. Parasitism is reduced at temperatures above 25°C. This parasite is also extremely susceptible to broad-spectrum chemical insecticides, especially synthetic pyrethroids. *Diadegma semiclausum* alone may not be adequate to give complete control of diamondback moth even in the highlands, because when temperatures exceed 30°C, the diamondback moth population increases, presumably due to the mortality of *D. semiclausum* adults. Under such circumstances, a few applications of *Bacillus thuringiensis* Berliner are essential to supplement the control achieved by the parasite.

Introduction

Vegetables have been an important part of Chinese diet for centuries. Twenty-eight major plant species are consumed as vegetables in Taiwan. Among the major plant species, crucifers are by far the most predominant group grown over 25% of the total hectarage planted to vegetables (PDAF 1988). These economically important vegetables are also the hosts of a large number of destructive insect pests such as diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), imported cabbageworm (*Pieris rapae* Boisduval) (Lepidoptera: Pieridae)), striped fleabeetle (*Phyllotreta striolata* F. (Coleoptera: Chrysomelidae)), cabbage looper (*Trichoplusia ni* Hübner (Lepidoptera: Noctuidae)), cabbage webworm (Hellula undalis F. (Lepidoptera: Pyralidae)), and aphids (*Myzus persicae* Sulzer, *Lipaphis erysimi* Davis, and *Brevicoryne brassicae* L. (Homoptera: Aphididae). DBM is by far the most destructive pest of crucifers in Taiwan and elsewhere in Southeast Asia.

DBM Problem in Taiwan

DBM was reported as a pest of crucifers in Taiwan over 80 years ago (Hori and Shiraki 1910). It was also mentioned as a pest of crucifers three decades later by Sonan (1942) and was considered potentially important in 1960, although its damage was quite low (Chang 1960;

Tao et al. 1960). In the mid 1960s, this insect was ranked the second most important, following pyralids on summer radish (Chen and Su 1986). Extensive insecticide screenings conducted in late 1960s indicates that DBM was already a serious problem (Ho and Liu 1969; Lee 1968, 1969; Tang 1967). From the initial two insecticides recommended for DBM control in Taiwan in 1965, the number of chemicals registered for this purpose rose to 8 in 1970, 17 in 1975, 25 in 1980, 31 in 1984 and 35 in 1989 (PDAF 1990). Practically every year, new chemicals are added, but the ineffective old ones are rarely dropped from the recommendation list.

Availability of the host-plant throughout the year, rapid turnover of generations under favorable tropical to subtropical conditions and intensive use of insecticides to combat this pest have resulted in DBM in Taiwan developing resistance to all chemical insecticides presently being used. As a result, the damage by this pest continues unabated. In some areas DBM threat has forced farmers to switch to other vegetable crops.

In view of the seriousness of the DBM problem in Taiwan and elsewhere in Southeast Asia, research at the Asian Vegetable Research and Development Center (AVRDC) has been focused on finding practical control measures to reduce dependence on chemical insecticides. The alternative controls that AVRDC explored are: (1) finding crucifer cultivars resistant to DBM (AVRDC 1981a, b), (2) cultural practices such as intercropping and overhead sprinkler irrigation that will reduce DBM infestation (AVRDC 1985, 1987), and (3) use of insect viruses and *Bacillus thuringiensis* Berliner (AVRDC 1975, 1976). These control measures, however, proved to have limited utility for the control of DBM on a sustainable basis. From 1985 onwards, therefore, AVRDC's research has focused on the introduction of parasites of DBM to help control this pest in Taiwan and, if successful, make similar attempts in countries in the region.

Parasite import and rearing

Diadegma semiclausum Hellen (Hymenoptera: Ichneumonidae), a larval parasite of DBM, is widespread in Europe (Hardy 1938; Voukassovitch 1927; Rusinov 1977; Mustata 1987) and is believed to be one of the parasites that is keeping DBM population under control in that continent. This parasite has been introduced into South Africa (Evans 1939), New Zealand (Robertson 1948), Australia (Waterhouse and Norris 1989), and Indonesia (Vos 1953) to control DBM. It has become established in certain areas of these countries. AVRDC imported *D. semiclausum* in 1985 from Indonesia (Talekar 1988) where it was introduced from New Zealand in the early 1950s (Vos 1953), and where it is now well established in the highlands (Sastrosiswojo and Sastrodihardjo 1986).

This parasite was reared on second instar DBM larvae raised on common cabbage seedlings maintained at 26 $\pm$ 2°C. The parasite pupae or adults from the routine rearing were utilized for research and field releases.

Parasitism study

Soon after importation, we conducted one field trial where common cabbage was planted in three 40 $\times$ 15 m parcels of land. Each parcel was enclosed on four sides and the top by fine mesh nylon net. Three weeks after cabbage transplanting, 250 DBM cocoons were introduced in the first two parcels and the third was maintained as a DBM-free check. Starting 1 week after DBM release, *D. semiclausum* adults were introduced periodically in one of the two cages where DBM was also introduced. Parasitism of DBM larvae was monitored periodically and cabbage yield was recorded at harvest.

Diadegma semiclausum readily infested DBM larvae. The average parasitism, which was only 13.1% about a month after the initiation of parasite release, reached 65.4% 6 weeks later just before harvest. Consequently cabbage yield increased significantly (Table 1). In fact, the yield was double that of control plot where only DBM was introduced. However, this yield was still significantly lower than in the DBM-free check. Obviously, the parasitism was not high and early enough to give complete control of DBM. Nonetheless, the experiment indicated

that *D. semiclausum* can infest DBM under Taiwan field conditions and thus has potential in DBM control on farmers' fields.

Table 1. Yield response of cabbage subjected to infestation by DBM with and without parasite.[a]

DBM status	Yield (t/ha)
Only DBM released	7.67 c
DBM + parasite	14.83 b
No insect release	30.93 a

[a]Planting date: 16 Jan. 1985, DBM released: 7 Feb. 1985, *D. semiclausum* released: 14 Feb. 1985 to 4 March 1985. Harvest date: 4 April 1985. Means followed by the same letters are not significantly different at 5% level according to Duncan's multiple range test.

Parasite release

Based on the results of the above experiment, attempts were made to introduce the parasite at three distinct agroecological areas of Taiwan where crucifers are grown. The first location, Luchu township, is only 10 m above sea level in Kaohsiung county (Fig. 1). The second location, Yangmingshan in suburban Taipei, is 700 m above sea level. The third area, Wuling near Lishan hill station, is in the central mountain range 1700 m above sea level. At Luchu, crucifers, mainly cauliflower and broccoli, are grown in the relatively cool dry season from October to April, and rice or other crops during the hot wet season from May to September. At Yangmingshan and Wuling, crucifers – mainly cabbage – are grown in summer, from May to September, and the land remains mostly fallow throughout the rest of the year. At the latter two locations, temperatures during December-January often dip to below freezing. At all three locations, DBM is endemic and has developed resistance to practically all insecticides presently being used in its control. At all three locations parasite release was supplemented by application of *Bacillus thuringiensis* Berliner.

At Luchu 35,166 *D. semiclausum* cocoons (emergence 75-80%) were released between October 1985 and April 1986 over a 15-ha area. Despite apparent suspension of chemical insecticide, *D. semiclausum* failed to parasitize DBM larvae and no adults were visible in the field. The native parasite *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae) was present and its parasitism reached 27%.

At Yangmingshan 35,600 *D. semiclausum* cocoons were released over a 10-ha area. During a weekly monitoring survey, only on three occasions was *D. semiclausum* found parasitizing DBM larvae; the parasitism ranged from 4.5 to 7.6%. *Cotesia plutellae*, however, parasitized 35-40% of DBM larvae.

At Wuling, about 35,000 *D. semiclausum* cocoons were released on a 17-ha area 2 weeks after cabbage transplanting in April 1986. Within 1 month, DBM parasitism reached 75%. After this, the DBM population was reduced so drastically that no further observations could be performed. There was no difference in the yield obtained during 1986 and the previous year, but the cost of insecticide used was reduced from NT$42,000 to 17,000/ha in 1986 (1 US$ = 36 NT$) and frequency of spraying from once a week to once every 9-10 days (Talekar 1990).

Parasite establishment

Among the three locations where *D. semiclausum* was released, Wuling is the only site where the parasite has become established (Fig. 1). A survey in 1988, 2 years after the introduction of *D. semiclausum*, revealed that 57% of the DBM population was parasitized by both parasites. *Diadegma semiclausum* parasitism accounted for 46% and *C. plutellae* 11%. In Lishan, which is 1600-2000 m above sea level and 20 km south of Wuling, *D. semiclausum* was present but the DBM pupulation was too low to determine the extent of parasitism. In Chingchin, 1600-2000 m above sea level and 60 km south of Wuling, 68% of the DBM population was parasitized.

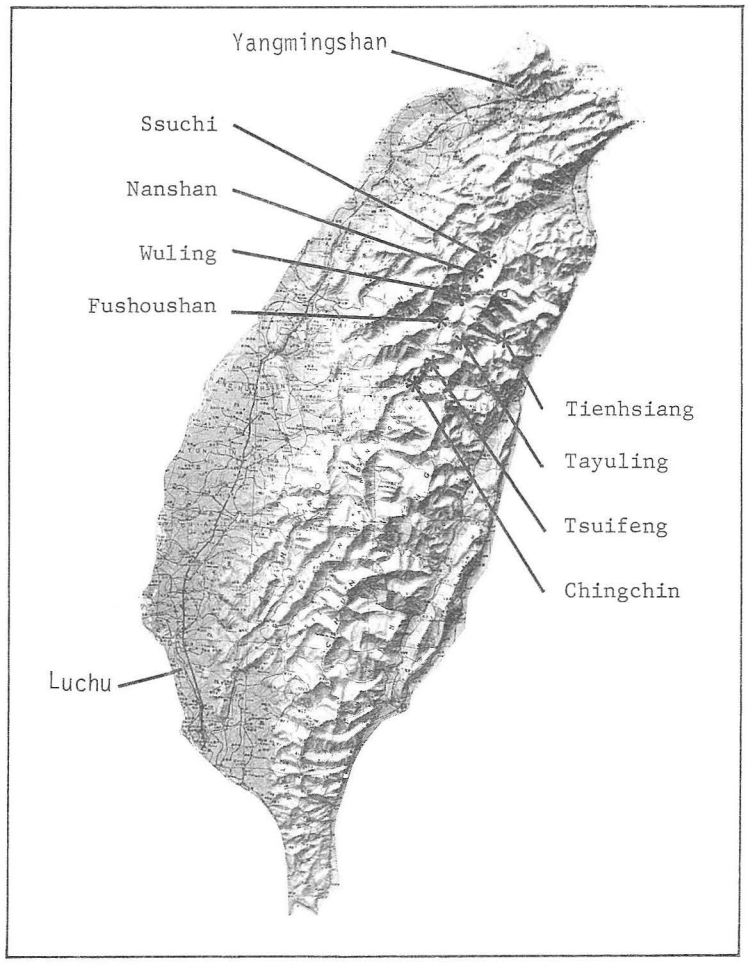

Fig. 1. Map of Taiwan showing *D. semiclausum* release and establishment sites.

Diadegma semiclausum accounted for 47% of the parasitism and *C. plutellae* 21%. In Tienhsiang, 1000 m above sea level and 30 km east of Wuling, *D. semiclausum* was present but the DBM population was too low to reliably determine the extent of parasitism. In Nanshan, 1100 m above sea level and 50 km north of Wuling, *D. semiclausum* was present. However, the DBM population was too low to reliably judge the extent of parasitism. In 1989 and 1990, *D. semiclausum* was present at all the above locations.

Diadegma semiclausum has now been established in crucifer-growing areas in the highlands of central Taiwan. All farmers in this area report considerably less DBM damage and consequently there is very little need for insecticide use. *Cotesia plutellae* occurred in the area in the past, but *D. semiclausum* was introduced in 1986 and has become well established and is spreading. The parasite can overwinter and has alternate hosts elsewhere (Hardy 1938). It is possible that *D. semiclausum* has alternate hosts in Taiwan, but no attempt was made to study them.

In our survey of parasitism in 1990, we also found *Diadromus collaris* Gravenhorst at all locations. *Diadromus collaris* was found in 1966 parasitizing DBM at Taipei in lowland Taiwan (Wu 1968). No further information on its parasitism in Taiwan is available. This is the first time *D. collaris* was found established in the highlands of Taiwan.

More than 1000 ha are grown to two crops of cabbage in the highlands every year. No reliable estimate of insecticide cost to control DBM before the introduction of *D. semiclausum* is available. However, based on Yangmingshan farmers' expenditure of NT$5,500/ha per crop, the parasite introduction represents savings of over NT$10 million (US$370,000) per year in insecticide cost alone. In addition, the reduced insecticide use lessened the amount of toxicant being washed off from the highlands into water streams and polluting the river water and coastal areas around Taiwan. Unfortunately, these benefits cannot be realized immediately because the vegetable farmers in Taiwan are habitual pesticide users and routinely apply prophylactic chemical sprays before the pest insect becomes sufficiently abundant to justify such treatment. This treatment kills the parasites, which increases the DBM population and subsequent damage.

Successful establishment of *D. semiclausum* in the highlands and failure in the lowlands indicates that temperature differences could be responsible. A laboratory study was therefore conducted to investigate optimum temperature for the parasitism of DBM by *D. semiclausum*. Second instar DBM larvae feeding on cabbage seedlings were exposed to *D. semiclausum* oviposition at 15, 20, 25, 30 or 35°C for 24 hours. All larvae were then maintained at 26±2°C until pupation, at which time the number of *D. semiclausum* and DBM pupae were recorded. Parasitism by *D. semiclausum* increased sharply with increasing temperature from 10 to 25°C (Fig. 2). It declined thereafter and at 35°C, the rate was lower than at 10°C. A temperature range of 15-25°C appears to be suitable for *D. semiclausum* parasitism of DBM larvae. Although the mean temperature is about 20°C during the peak crucifer-growing season in all three areas of Taiwan when DBM is more likely to cause damage, the range of temperature at Wuling (10-27°C) is much more favorable than the one at Yangmingshan (13-30°C) or Luchu (15-32°C) for the parasitism of DBM by *D. semiclausum*. We observed that *D. semiclausum* adults are highly sensitive to temperature beyond 28°C and die if held above 30°C.

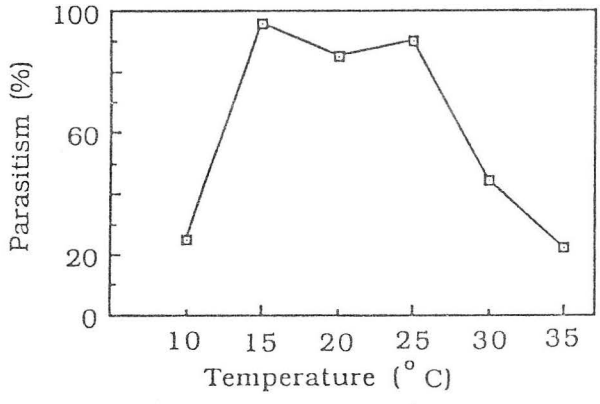

Fig. 2.
Effect of temperature on the parasitism of DBM larvae by *D. semiclausum*.

Inundative release of *D. semiclausum* in the autumn-winter crucifer season at AVRDC, where temperatures are similar to Luchu but where insecticide use is restricted, gives adequate control of DBM despite occasional high temperatures. Failure of similar control of DBM on farmers field in lowland Luchu appears to be due to the use of broad-spectrum chemical insecticides despite the failure of these chemicals in giving satisfactory control of DBM. Our laboratory test with *D. semiclausum* adults showed that the adults are extremely sensitive to synthetic pyrethroids such as deltamethrin (Table 2). Recently introduced benzoylphenylurea insect growth regulators, selective aphicide pirimicarb and *B. thuringiensis* are much less toxic to *D. semiclausum*. These insecticides, however, were relatively less harmful to puape of *D. semiclausum* possibly due to the barrier of cocoon material that covers the puape. The indiscriminate and habitual use of broad-spectrum synthetic insecticides by lowland farmers appears to be a major obstacle to the control of DBM by innudative release of *D. semiclausum*

Table 2. Toxicity of selected insecticides to *D. semiclausum*.

Insecticide (concentration)	Mortality (%)[a]	
	Adults[b]	Pupae[c]
B. thuringiensis (0.1% product)	32.5 ± 25.3b	26.7 ± 25.2a
Teflubenzuron (0.0075% AI)	17.5 ± 22.6b	33.3 ± 15.3a
Pirimicarb (0.05% AI)	11.3 ± 8.5bc	26.7 ± 20.8a
Deltamethrin (0.01% AI)	100a	40.0 ± 17.3a
Water	3.8 ± 4.8c	23.3 ± 20.8a

[a]Data are means (± SD) of four replicates. Means in each vertical column followed by the same letter are not significantly different ($P > 0.05$, Duncan multiple range test). [b]Observations taken 3 days after insecticide application. [c]Mortality was judged at the emergence of adults from the pupae.

in the lowlands. In insecticide-restricted areas where inundative release of *D. semiclausum* gives adequate control of DBM, routine sprays of broad-spectrum chemicals on crops in the neighboring fields do not reduce the parasitism of DBM by *D. semiclausum* (AVRDC 1990).

Even in highland areas where *D. semiclausum* is established, occasional surges in temperature above 30°C are detrimental to the survival of *D. semiclausum* adults. Soon after such rises in temperature parasitism is reduced and DBM populations rise. Under such circumstances, spraying of selective insecticides like *B. thuringiensis* or an insect growth regulator becomes essential.

We also observed that the rate of *D. semiclausum* parasitism goes up as the season progresses, whereas that of *C. plutellae* parasitism, which is always high at the beginning of the season, goes down as the season progresses (Fig. 3). Introduction of *C. plutellae* in areas where *D. semiclausum* is being introduced will complement the DBM control by *D. semiclausum* and vice versa.

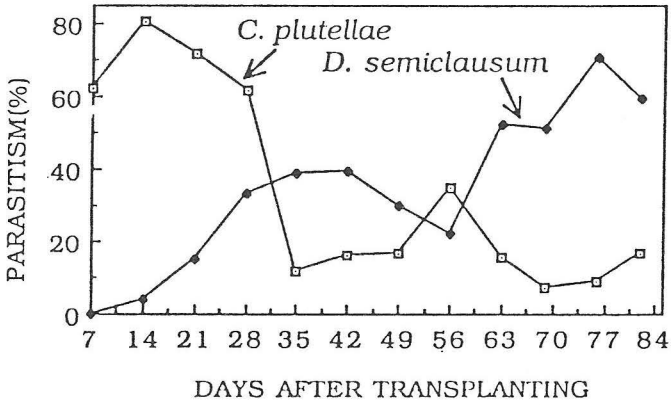

Fig. 3. Parasitism of DBM larvae by *D. semiclausum* and *C. plutellae* in one cabbage crop season.

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Diamondback Moth in the Philippines and its Control with *Diadegma semiclausum*

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Abstract

Diamondback moth, *Plutella xylostella* (L.), is the most destructive pest of crucifer crops in the Philippines. Life cycle, population dynamics and biological control with the larval parasitoid *Diadegma semiclausum* have been studied (1988-90) in the mountains of Northern Luzon. One generation of the diamondback moth requires 24.7 days, therefore about 15 generations of the pest may occur during 1 year. The average life expectancy of the females is 16.7 days, during which an average of 233 eggs (maximum 639) are deposited. The continuous monitoring of the diamondback moth field population with light and pheromone traps proved that during the rainy season (June-October) only 5-10 moths/week were found; during population peaks (January and February) almost 3000 moths/week were found. Only one natural enemy — *Cotesia plutellae* (Kurdjumov) has a certain importance in controlling the pest. The parasitism reaches 70% but its efficacy is reduced by hyperparasitism to 80% by a group of six hymenopterans. Starting in March 1989 *D. semiclausum* was imported from Taiwan. The performance of *D. semiclausum* was evaluated in field experiments with screen-covered cabbage plots. The parasitism reached 95%, and in two of three experiments the yield of cabbage was significantly higher than in the control. In an open-field experiment the parasitoid was established with rates of parasitism from 12 to 15% increasing to 64% at harvest time.

Introduction

Crucifer crops like cabbage, *Brassica oleracea* L., Chinese cabbage, *Brassica campestris (L.)* ssp. *pekinensis*, and radish, *Raphanus sativus* L., are the most important vegetables in the Philippines after tomato and eggplant (Bureau of Agricultural Statistics 1988). In 1986 an area of 121,000 ha was planted nationwide with these vegetables (Valmayor and Tiamzon 1988). Cabbage is grown mainly in the mountains of Northern Luzon and is an important source of income for the people.

Diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), is the most destructive pest of cabbage. DBM ranks above a group of pests with only seasonal importance, e.g. fleabeetle *Phyllotreta* sp., aphids *Lipaphis erysimi* (Kalt.) and *Myzus persicae* (Sulz.) (Homoptera: Aphididae), cutworm *Spodoptera litura* (F.) (Lepidoptera: Noctuidae), tomato fruitworm, *Helicoverpa armigera* (Hb.) and cabbage looper *Trichoplusia ni* (Hb.) (Lepidoptera: Noctuidae), and cabbage butterfly *Pieris canidia* L. (Lepidoptera: Pieridae).

In Southeast Asia DBM is highly resistant to most insecticides used for its control (Perng and Sun 1987). In Thailand and Malaysia resistance to synthetic pyrethroids occurred after 1-2 years (Cheng 1988). In the Philippines growth-regulator insecticides (based on teflubenzuron) was successful for about 2 years. A reduced efficacy of even *Bacillus thuringiensis* Berliner is reported from the DBM strain of North Luzon (Theunissen 1981; Kirsch and Schmutterer 1988).

The farmers are using mainly organophosphorus insecticides (triazophos, profenofos, methamidophos), pyrethroids (fenvalerate and deltamethrin) and carbamate insecticides (bendiocarb) for its control. The average number of insecticide sprayings per crop is 12, but could go as high as 20. In some areas farmers are giving up growing cabbage because of their inability to control DBM.

In the Philippines DBM has only one natural enemy of some importance, the larval parasitoid *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae). Field parasitism by *C. plutellae* varies over locations, years and seasons from 1 to 70%. Besides the overuse of insecticides as a limiting factor, there is a group of at least six hyperparasitoids limiting *C. plutellae* populations up to 90%. These secondary parasitoids were identified as *Tetrastichus coeruleus* (Bingham) (Hymenoptera: Eulophidae) (LaSalle 1990), *Aphanogmus fijiensis* (Ferriere) (Hymenoptera: Ceraphronidae) (Polaszek 1989), *Diatora* sp. (Hymenoptera: Ichneumonidae) (Fitton 1990a; Horstmann K. pers. comm.) and three species of the genus *Trichomalopsis* (Hymenoptera: Pteromalidae) (LaSalle 1990): these are *T. apanteloctena* (Crawford), *T. ?deplanata* Kamijo & Grissell and *Trichomalopsis* sp. In DBM rearings based on collections from the field the pupa parasitoid *Itoplectis* sp. (Hymenoptera: Ichneumonidae) (Fitton 1990) was also found. Occasionally, another larval parasitoid of the genus *Diadegma* (Hymenoptera: Ichneumonidae) was found. This species of *Diadegma* is unknown in Europe and not yet been identified (Horstmann K., pers. comm. 1990).

Diadegma semiclausum plays an important role in the control of DBM in Taiwan (AVRDC 1985) and in Indonesia (Sastrosiswojo and Sastrodihardjo 1986). In March 1989 the Philippine-German Biological Plant Protection Project imported from Taiwan some 100 *D. semiclausum* to evaluate the potential of this parasitoid as a biocontrol agent under Philippine conditions, first in the laboratory, semi-field and then in field experiments. Before working with parasitoids the life cycle, population dynamic, density and occurrence in the course of the year were observed in this region.

Material and Methods

Field observations of the DBM and semi-field experiments were carried out at the experiment farm of the Benguet State University in La Trinidad, Philippines, which is located 250 km north of Manila in the mountains of Northern Luzon (about 1300 m above sea level). *D. semiclausum* was released into the field in La Trinidad Valley, Baguio (about 1500 m above sea level) and Sayangan (about 1700 m above sea level).

Laboratory experiment

Six hundred DBM larvae were measured, and their length, width and diameter of the head capsule recorded. On the same day, eggs of DBM laid on 8-week-old cabbage plants were counted. For a period of 15 days, 40 larvae were taken daily, killed and measured. When a sudden increase in the head capsule diameter was observed a molting to the next instar was assumed. For each instar a range in the head diameter was determined and the age of the larvae led to the duration of that instar. The average temperature was 19.5°C (range 15-25°C). The relative humidity was 90% with a range of 70% (day) and 95% (night). To compute the effective thermal total, the threshold of 8.5°C from Yamada and Kawasaki (1983) was adopted.

For laboratory studies on DBM and the parasitoids, glass cages were used. Four-week-old potted cabbage plants were offered to newly hatched single pairs of DBM adults for oviposition. The plants were exchanged daily and the eggs were counted. Unmated pairs were exchanged. The moths were fed with a 30% honey:water solution offered in soaked cotton.

Semi-field and field experiments

Population dynamic of DBM was observed in a 700 m^2 survey area planted with about 2500 cabbage plants. A light trap and one pheromone trap was placed in this field. The light trap

was an ordinary 100-W bulb. Tests with a special UV black-light produced similar results. Moths were caught from 6 p.m. to 6 a.m. A killing solution of 200 ml 3% formaldehyde was put in a plastic bottle. Every day the bottle was changed and the insects were counted. The sticky insert of the pheromone trap was changed weekly, and the pheromone lure every 2 weeks.

Cabbage was sown in seedbeds and transplanted after 30 days into the field in plots of 1.3 × 5.5 m. Each plot consisted of about 40 cabbage plants in three rows with a planting distance of 35-40 cm. Insect counts were done on six plants/plot taken from the middle row. For semi-field experiments eight plots were covered with nylon screen. The screen was placed 3 days after transplanting. The natural infestation with DBM by that time was sufficient for the experiment. The height of the cages was 2 m. To support the insects two yellow carton papers/cage smeared with a food medium were mounted on the screen at a height of 20 cm.

Release experiment with *D. semiclausum* in the field was conducted from February to May in a broccoli plantation at the Puyat farm in Baguio City. *B. thuringiensis* was sprayed in the first weeks after transplanting.

The degree of parasitism was calculated as follows:

$$\% \text{ parasitism} = \frac{\textit{Diadegma} \text{ cocoons}}{\textit{Diadegma} \text{ cocoons} + \textit{Cotesia} \text{ cocoons} + \text{DBM pupae}} \times 100$$

Results

DBM in Northern Luzon

DBM is closely associated with cabbage. In the valley of La Trinidad, cabbage is grown during the dry season, i.e. from October/November to May/June. No important alternate host plant was observed as a food source for DBM.

Population dynamics

The DBM was present throughout the year (Fig. 1), even during the peaks of heavy rain. In the rainy season it was caught at the rate of 5-10 adults/week in the pheromone trap and 2-3 adults/night in the light trap. During population peaks in January and February 500-700 moths/night were counted in the light trap and 200 moths/week in the pheromone trap.

The DBM population is not primarily suppressed by the reduction of cabbage cultivation in May and June but rather by the rain. The rain induces various mortality factors, such as physical damage by rain drops or fungal infection due to high humidity.

The changing importance of the two traps with respect to the number of moths they caught is caused by weather changes. The effectiveness of a light trap depends very much on the temperature during the night. In the mountains of northern Luzon the coldest period of the year is November-January. Night temperature goes as low as 0°C. The light trap catches shown in Fig. 1 constitute the weekly average. During the rainy season both traps caught more moths of the cutworm *S. litura*.

Life cycle

The developmental time for each instar and the head sizes of the larvae are shown in Table 1. These data correspond well with the findings of Yamada and Kawasaki (1986). Regarding fitness and multiplication, the Philippine conditions with 20°C and 90% RH are very favorable for DBM. Dusk – some 30 min between 6.30 p.m. and 7.30 p.m. – is the adults' most active time for mating and oviposition. In this period the thermohydrograph recorded 20-21°C and 90-95% RH. These are the optimum conditions for DBM. The total development time of 23.7 days suggests, in theory, 15.4 generations/year are possible. However, considering the range

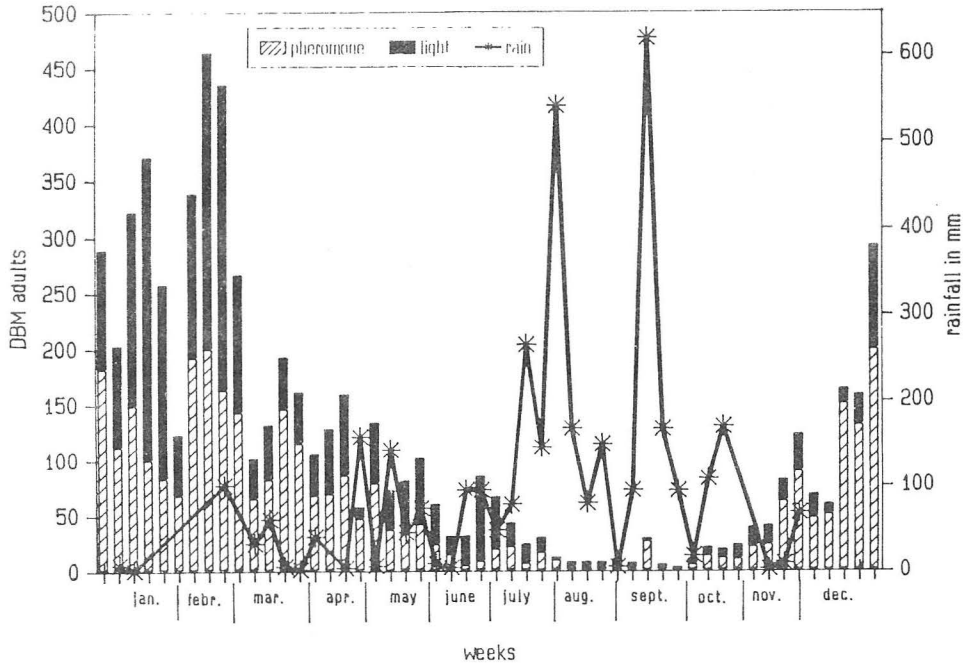

Fig. 1. Monitoring of DBM population by light trap and pheromone trap and rainfall for one year. La Trinidad (Philippines), 1989. Light trap data were taken daily, pheromone trap data weekly.

Table 1. Life cycle of DBM in Northern Luzon (Philippines)[a].

Observations	Development stage							
		Larval stages						
	Egg	L1	L2	L3	L4	Prepupa	Pupa	Adult
Head capsule[b] (mm)	–	0.14 (0.10-0.18)	0.23 (0.20-0.30)	0.42 (0.33-0.48)	0.57 (0.50-0.65)	–	–	–
Life span[c](days)	4.17 ±0.95	4.18 ±0.57	1.83 ±0.45	3.81 ±0.54	3.33 ±2.09	1.17 ±0.94	5.16 ±0.78	16.10[d] ±9.43
Time to reach this stage (days)	–	4.17	8.35	10.18	13.99	17.32	18.49	23.65
Day-degree °c[e]	46	46	20	42	37	13	57	Σ=261[f]

[a]Field observations (temp.: 19.5°C, RH: 90%); [b]mean (range); [c]mean ± standard deviation; [d]life expectancy of females (laboratory observation); [e]threshold temp.: 8.5°C; [f]sum of all developmental stages.

of especially the last three instars (4th, instar lavae, prepupa and pupa) expressed here in standard deviation it becomes obvious that generations overlap. After two generations the individuals found on the plants in the field cannot be accurately assigned to a certain generation. The head capsule sizes are almost the same as those measured by Harcourt (1986). The measurements of length and the width of the larval bodies are useless because of its wide range. The measurements overlap from the 2nd to the 3rd and from the 3rd to the 4th instar.

A female DBM can lay 233 eggs in 12 days. The peak of oviposition is the 2nd day, and after 6 days 90% of the total number of eggs are deposited. The longevity of the females and their fecundity are positively correlated ($R^2 = 0.74$). The results shown in Table 2 are similar to those of Ho (1965) who found an average of 230 eggs/female and Bhalla and Dubey (1986) with 243 eggs/female. However, the maximum number of eggs/female is less in the other studies except Salinas (1986).

Control of DBM with *D. semiclausum*

The pest populations of *D. semiclausum* treated plots and of control plots are given in Fig. 2. The initial pest population was the same in all eight plots. Twenty days after the first release the cocoons of the F_1 generation of *D. semiclausum* were found. Parasitism increased continuously reaching 95%.

An important growth stage for cabbage regarding yield is the period of early head formation. Protecting the plants against pest attack in this span from 45 to 65 days after transplanting can prevent later yield losses. Using *D. semiclausum* it was possible to keep the DBM infestation in this critical time (57 days after transplanting) as low as 5-7 larvae/plant.

Table 2. Reproduction and fecundity of DBM in laboratory observations.

Observations	Mean	Range
Pre-oviposition period (days)	0.3 ± 0.68	0-2
Oviposition period (days)	11.8 ± 6.21	4-20
Post-oviposition period (days)	4.6 ± 4.90	0-16
No. of eggs per female	232.7 ± 193.94	38-639
No. of eggs per female per day	15.9 ± 7.70	2-30
Max. No. of eggs per female per day	66.2 ± 31.36	26-130
Life expectancy of females (days)	16.7 ± 9.43	4-27

(n = 10; Temp.: 19.5°C; RH: 90%)

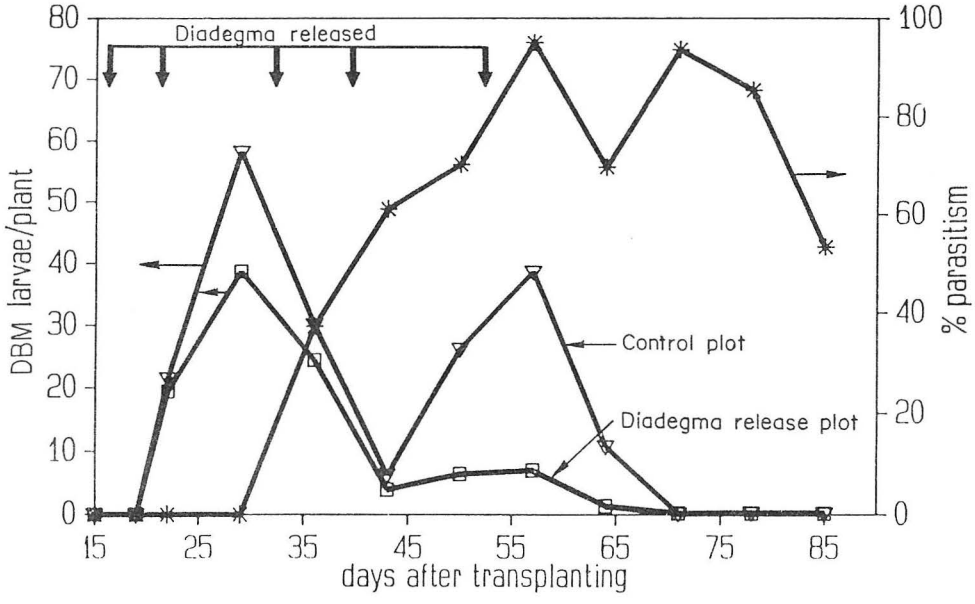

Fig. 2. Population of DBM in control plot and *D. semiclausum* released plots. Both plots were under screen cages in a cabbage field. Swamp, La Trinidad (Philippines), 1990.

The plants in the control plots, however, had up to 40 larvae/plant. This difference is significant (DMRT, $P = 0.05$) and is responsible for the differences in the harvest as given in Table 3. The mean number of larvae/plant over all 10 counts is 18.7 in the control and 9.8 in *D. semiclausum* plots (DMRT $P = 0.05$)

A field release of *D. semiclausum*, with close monitoring of the population, was conducted from February to May 1990 in Baguio. One hundred and fifty pairs of *D. semiclausum* were released 22 days after transplanting in a broccoli garden surrounded by hedges and bushes. DBM pupae and *Diadegma* cocoons were counted to compute parasitism. Fifteen days after release the cocoons of the F_1 generation were found. The parasitoid was established and parasitism reached 64% at harvest time (Fig. 3). This experiment was done under special conditions, since the pest pressure did not compare to other crucifer plantations in Baguio or La Trinidad at that time.

Comparing cabbage under *Diadegma* release with untreated cabbage the weight/cabbage head was higher and, thus, the yield/plot. The single plant weight was recorded after cleaning the cabbage to a marketable condition. The difference in the attributed weight/head results directly from the pest attack and indirectly by removing the damaged cover leaves. Each head of cabbage was classified but usually all plants of a treatment were in the same quality class. The three classes of quality — A, B and C — applied in the vegetable market of the Philippines are reflected in a return difference of 1-2 Pesos (4-8 US cents)/kg.

Table 3. Harvest of three control experiments where *Diadegma* was used for DBM control in cabbage (La Trinidad, Philippines, 1989-90).

Crop attribute	Experiments (location)		
	Balili 1	Balili 2	Swamp
Yield (kg/plot)			
Diadegma	5.59 a	21.60 a	27.28 a
Control	2.45 a	9.30 b	8.60 b
Weight of cabbage (kg/head)			
Diadegma	0.41 a	0.81 a	0.87 a
Control	0.35 a	0.46 a	0.56 b
Quality of cabbage (class)[a]			
Diadegma	B	A	A
Control	B	B	C

Data followed by the same letter are not significantly different (LSD $P = 0.05$). [a]Classes of quality: A = no damage, B = 3 to 5 leaves are removed, C = 5-10 leaves are removed.

Conclusions and Prospects

DBM was successfully controlled with the release of *D. semiclausum*. If it were possible to produce and release the necessary numbers of *D. semiclausum* this system could be transferred to the complete vegetable area of northern Luzon. But the sole import and release of some thousands of *D. semiclausum* to establish this new species is not sufficient. *D. semiclausum* is quickly included in the balance of the ecosystem. It is attacked by the same group of hyperparasitoids as *C. plutellae*. In Baguio a few weeks after release of *D. semiclausum*, 30% of secondary parasitoids emerged from collected *Diadegma* cocoons. It is uncertain whether the established populations of the beneficial species such as *Diadegma* and *Cotesia* are able to suppress the pest population to an acceptable level without any insecticides. But monitoring the pest and the beneficial species could reduce insecticide application. The natural balance of DBM and beneficials could work to a certain level of pest infestation; beyond this level insecticide application will be necessary.

It is shown that the cycle of the DBM generations is never broken throughout the year. If farmers could adjust their rotations not to grow cabbage or other crucifer crops for a certain

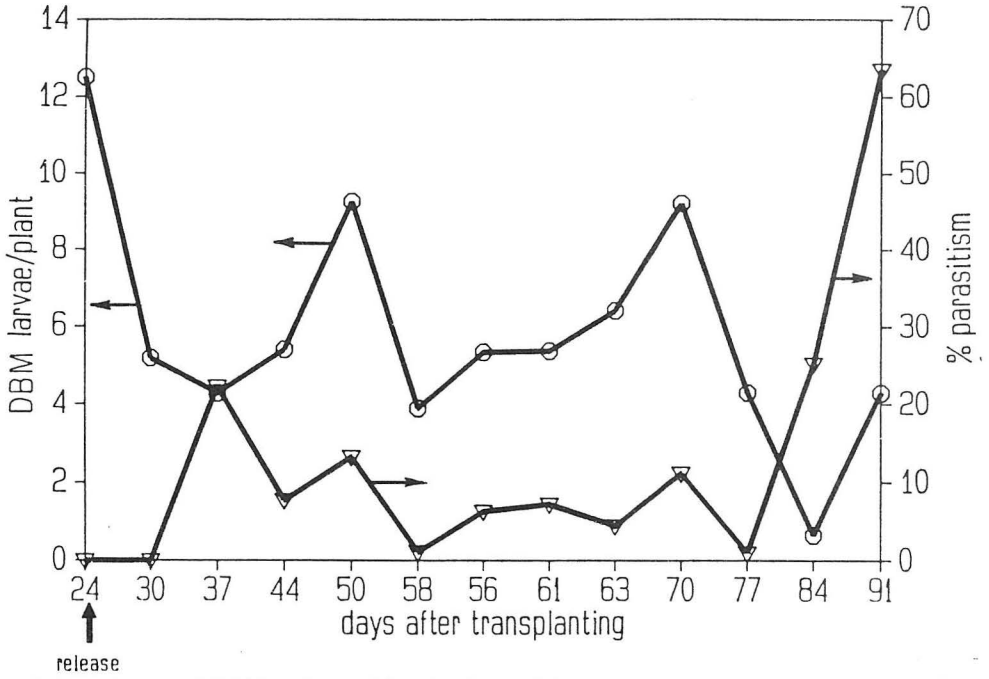

Fig. 3. Population of DBM and parasitism by *D. semiclausum* in open field. Puyat, Baguio City (Philippines) 1990.

time in the year during the rainy season for instance the DBM population cycle would break down. A new immigration and population increase of the pest should be attacked by a release of the parasitoids to accelerate the buildup of the initial population of these beneficials.

Other activities, like threshold spraying, adjusted crop rotation, destruction of plant residues after harvest and the use of selective insecticides are neccessary. Nevertheless, the beneficials would be more effective if they were better protected and supported. Sowing flowering plants such as flowering pechay around the plot some weeks before transplanting the cabbage could be one way to attract and feed the beneficials.

Acknowledgments

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Management of Diamondback Moth with *Cotesia plutellae*: Prospects in the Philippines

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Abstract

An indigenous *Cotesia plutellae* Kurdjumov was recorded for the first time in 1982 in Baguio City, Benguet and Mountain Province. The low level parasitism (1.9-16.5%) of diamondback moth (*Plutella xylostella* (L.)) by this parasite is due to the hyperparasite (*Trichomalopsis* sp.), and the entomophagous fungus, *Erynia* sp. and frequent insecticidal applications. Indigenous *C. plutellae* was absent in the lowland cruciferous farms in Laguna, Cavite, Misamis Oriental and Bukidnon from August 1989 to July 1990. Three to four releases of *C. plutellae* imported from Taiwan at the rate of 3000/release/ha and integrated with 1-2 sprayings of *Bacillus thuringiensis* Berliner based on economic threshold level (ETL) reduced the population of diamondback moth in two successive plantings in Nagcarlan. The average parasitism was 17.4% and 36.5% in the first and second planting, respectively. Parasite cocoons were found in the nearby fields where the parasite was not released. The demonstration field is being maintained while other demonstration fields are to be set up in other towns. Our available data indicated that *C. plutellae* can be integrated with *B. thuringiensis* in controlling diamondback moth. The establishment of *C. plutellae* could be delayed by hyperparasite and infection by *Erynia* sp. Therefore, there is a need to introduce other parasitoids to supplement *C. plutellae*, preferably ones that could also parasitize *Crocidolomia binotalis* (Zeller).

Introduction

Cruciferous vegetables, cabbage (*Brassica oleracea* var *capitata* L.), petchay (*Brassica campestris* var. *chinensis*), raddish (*Raphanus sativus* L.) and mustard (*Brassica juncea* L.) are economically important in the Philippines. The area devoted to these vegetables in 1986 was 14,400 ha with a production of 135,130 t (Valmayor and Tiamzon 1988). Other crucifers grown are broccoli (*Brassica oleracea* var. *botrytis* L.) and cauliflower (*Brassica oleracea* var. *botrytis* L.).

One of the constraints in the production of cruciferous vegetables is the infestation of a group of insect pests; diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), the cabbage moth (*Crocidolomia binotalis* L.), the flea beetles (*Phyllotreta* spp.), common cutworm (*Spodoptera litura*), aphids and others. These insects greatly reduce both yield and quality of the produce.

DBM is the most important limiting factor in the production of cruciferous vegetables in the Philippines. The larva feeds on the foliage from seedling to harvest causing 100% yield loss if not controlled. Farmers rely mainly on chemical pesticides for its control resulting in the development of resistance to practically all insecticides used. The heavy usage of pesticides

kills the predators and parasites which used to contribute to the reduction of the DBM population and other pests. The destruction of the natural enemies causes the resurgence of the minor pests. These problems are becoming more severe each year, hence, restoration of the active role of biocontrol agents is seen as one way to minimize the present dependence on chemical pesticides.

Cotesia plutellae Kurdjumov (Hymenoptera: Braconidae), a solitary and DBM-specific larval parasitoid is found in subtropical and tropical countries. Results of various studies in other countries showed that the parasitoid disrupts the population of DBM in the field (Lim 1982; Feng and Wang 1984). In Taiwan it regulates the DBM population by supplementing with *Bacillus thuringiensis* var. *kurstaki* (AVRDC 1990).

This paper will discuss the presence of indigenous *C. plutellae* and its potential as a component in the management of DBM in selected lowland and mid-elevation areas in the Philippines.

Presence of Indigenous *C. plutellae*

Velasco (1982) documented the presence of *C. plutellae* for the first time in Baguio City and La Trinidad, Benguet, at an elevation of 1800 m. Poelking (1989) found this parastioid in Atok, Mountain Province. Velasco (1983) assessed its field performance along with that of fungus *Erynia* sp. in Baguio where it has so far been reported. Parasitization rate in the field ranged from 1.9 to 16.5% in the Velasco (1983) study, whereas in Poelking's (1989) study it was 0.2-50% in Benguet. Velasco attributed the low parasitism of *C. plutellae* to the presence of a hyperparasite (*Trichomalopsis* sp.) and the parasitic fungus *Erynia radicans* (Bufeld), and to the frequent insecticidal applications.

A survey was conducted from August 1989 to July 1990 to determine the presence of indigenous *C. plutellae* in selected lowland and mid-level crucifer-growing areas (Fig 1). Fifty cabbage plants at various locations were examined and the number of *C. plutellae* cocoons recorded. Likewise, 50 fourth instar DBM larvae were collected randomly from the field and reared in the laboratory. The number of parasitoid cocoons recovered from the culture was noted.

No parasitoid cocoons were collected or recovered from the cabbage/petchay farms in Laguna (Nagcarlan, Liliw, Majayjay, Los Baños, and Cabuyao), Cavite (Silang and Tagaytay), Misamis Oriental (Claveria) and Bukidnon (Lantapan). These areas showed heavy populations of DBM. Generally, plantings during May-September were less infested by DBM than those planted from November to March.

The use of insecticides is the only means utilized by farmers to control this pest. Most of them spray their cabbage/petchay at least once a week and even 2-3 times a week if the DBM population is extremely high for a total of 10-16 times/cropping. The repetitive and heavy application of insecticides employed by farmers appears to be the main reason why the indigenous *C. plutellae* was not encountered in the surveyed farms.

Importation and Mass Production

Taiwan strain of *C. plutellae* was introduced from AVRDC and reared at the Department of Entomology, University of the Philippines at Los Baños (UPLB), Philippines.

The distinguishing characteristics of *C. plutellae* from Taiwan and the local strain are: the Taiwan strain is yellowish whereas the local strain is dirty white and the size of the former is 3.1 mm while that of indigenous one is 2.5 mm. Based on Poelking's (1989) laboratory study, the imported strain also gave better parasitism (17.3-40%) compared to the local strain (13.8-33%).

The imported parasitoid was mass-reared for at least one generation (F_1) before being released in the field. Based on the series of mass-rearing, the developmental period of the imported parasitoid in the laboratory ranged from 7 to 10 days (egg to pupae). This is similar to the report of Lim (1982) of an average period of 9 days. Presently, the mass production/rearing capacity is only 1000-2000 cocoons/week but it has to be increased to expand the release area.

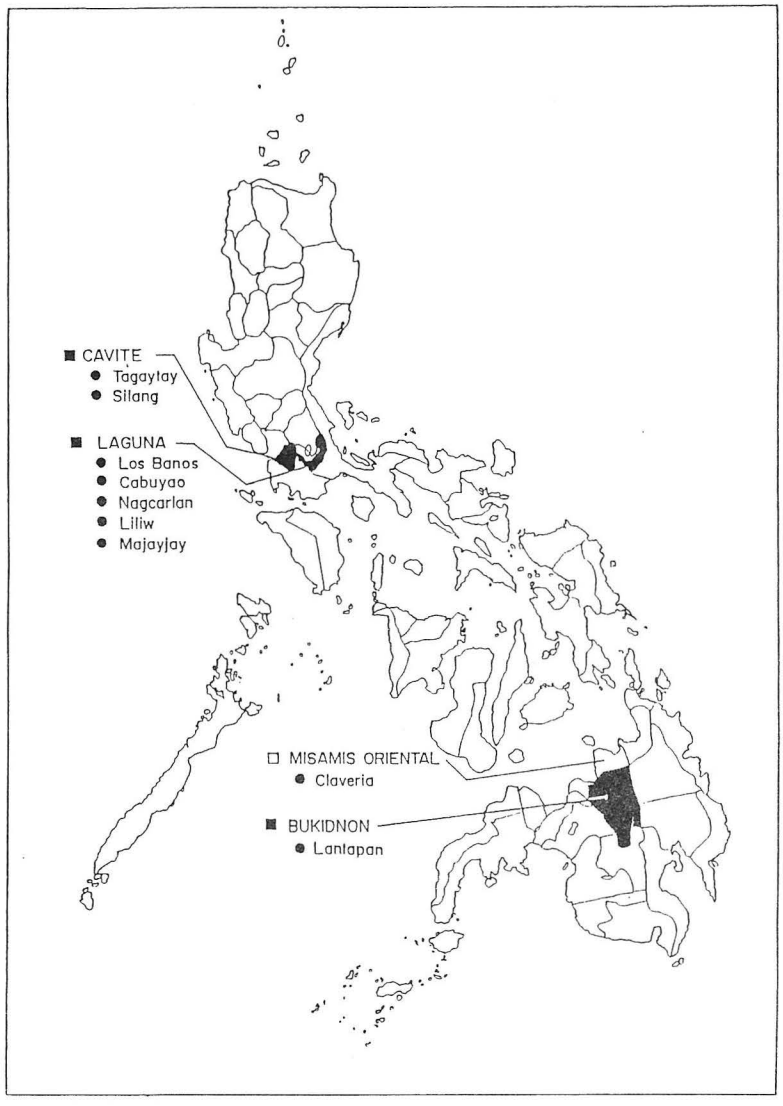

Fig. 1. Areas surveyed for indigenous *C. plutellae*.

Our problems in mass-rearing are: diseases, and abnormal development of the parasitoid and DBM host because of continuous rearing. These may affect the vigor and activity of the parastitoid. To minimize these problems, the culture stocks of the host (DBM) and the *C. plutellae* are periodically changed by new stocks, collected from the release areas or imported from AVRDC. On the other hand disease infection was prevented through proper sanitation in the laboratory.

Field Releases of Taiwan Strain *Cotesia plutellae*

A demonstration field is located at Nagcarlan, Laguna (Fig. 2), at an elevation of 600-800 m. The temperature ranges from 15 to 30°C, the lowest during November-January and the highest

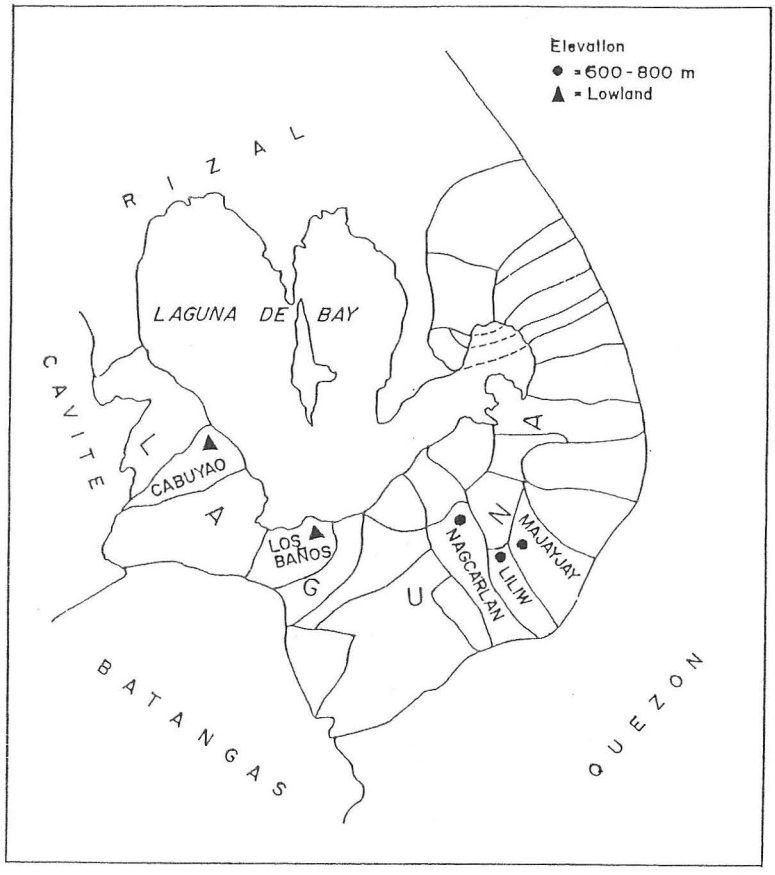

Fig. 2. The parasitoid release area in Laguna. Existing area Future.

during March and April. Cruciferous vegetables are grown sporadically and the area devoted to these crops is about 216 ha/year, including the nearby towns of Liliw and Majayjay (PAO 1990). They are planted in the traditional growing areas or newly cleared forest throughout the year. However, more cabbage is planted during October-February than in other months.

The 1000-2000 m^2 demonstration field was set up at Barangay Bukal, Nagcarlan (Fig. 3). The field was planted with cabbage (Scorpio and Kabuko) for two croppings. The cocoon or adult parasitoids were released starting at 3-4 weeks after the first planting and ending about 8 weeks after the second planting. Three and four releases were made during the first and second plantings, at the rate of 3000 cocoons/release/hectare. *Bacillus thuringiensis* was applied when the population of DBM increased above the recommended economic threshold level (more than 2 (3rd or 4th instar) larvae/plant at early stage and more than 5 (3rd or 4th instar) larvae/plant before heading).

The population buildup and parasitism of the introduced *C. plutellae* were monitored three weeks after the first release. Population buildup was determined by examining 50 plants with cocoons at 10-15-day intervals. For parasitism, 50 fourth instar DBM larvae were collected and reared in the laboratory. The number of parasitoid cocoons recovered were expressed in percent.

From the 2000-2500 cocoons introduced in the first planting (demonstration field) a total of 62 cocoons were recovered from six samplings but only two cocoons from farmers' fields (Table 1). The average parasitism in the demonstration field was 17.4% while in farmers' fields

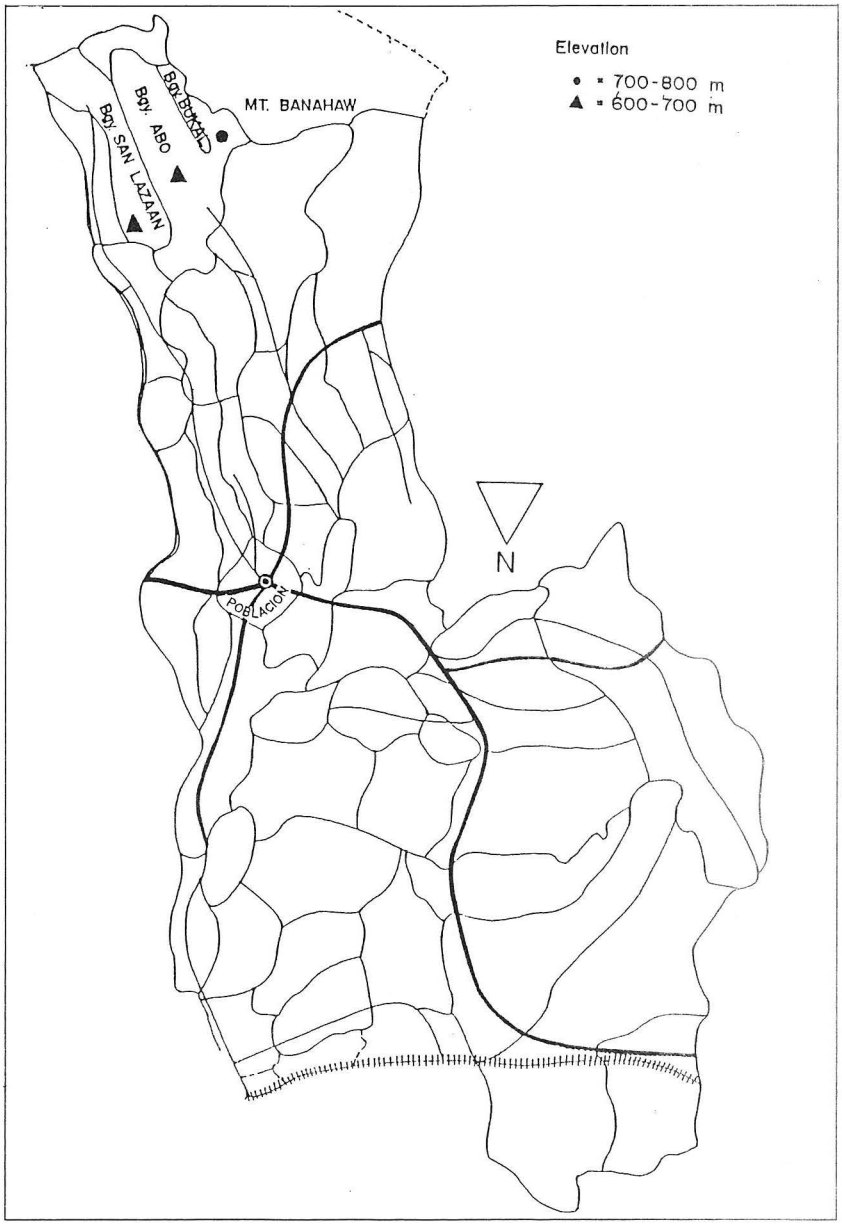

Fig. 3. The parasitoid release area in Nagcarlan. ● Existing area ▲ Future.

it was 0.7%. The low parasitism in farmers' fields was attributed to the intensive application of broad-spectrum insecticides.

Relatively more cocoons were recovered from the four releases during the second planting both from demonstration and farmers' fields. From the 2800 to 3000 cocoons introduced into the demonstration field, 140 and 134 cocoons were recovered from demonstration and farmers' fields, respectively. The total number of collected cocoons from both fields was almost the same at this planting but the average parasitism was still higher in the demonstration field with 36.5% but only 17.4% parasitism from farmers' fields. In the second planting, the number of cocoons

Table 1. Number of *C. plutellae* collected from cabbage fields at Nagcarlan, Laguna.

Observation date	Cocoons/50 plants and % parasitism			
	Demonstration field	% parasitism[a]	Farmers' field[b]	% parasitism
	First Planting (8 Oct 1989)			
09 Nov 89*	0	0	0	0
21 Nov 89*	4	4.0	0	0
01 Dec 89*	6	16.3	0	0
15 Dec 89	14	20.8	0	0
22 Dec 89	6	20.8	0	0
03 Jan 90	32	42.5	2	4.2
Total	62	17.4	2	0.7
	Second Planting (28 Dec 1989)			
16 Jan 90*	6	32.6	4	10.4
22 Jan 90*	30	53.2	24	8.0
01 Feb 90*	42	30.0	34	17.8
15 Feb 90*	34	32.0	37	26.7
01 Mar 90	28	35.0	35	24.0
Total	140	36.5	134	17.4

*Date of parasitoid releases (700 cocoons/release).
[a]Based on direct counting (50, 4th instar larvae) from the field. [b]200-300 m away from the demonstration field.

collected increased to 134 from three adjacent farmers fields 200-300 m away from the release site, compared to two cocoons in the first planting.

These farmers switched from chemical insecticides to *B. thuringiensis* during the second planting after seeing our demonstration plots. However, the parasitism in the farmers' fields was still lower than the demonstration field because of their intermittent *B. thuringiensis* sprayings. The DBM and *C. plutellae* populations and parasitism from the first planting (October 1989) to the third planting (March 1990) are shown in Fig. 4.

Generally, DBM populations in the demonstration field was lower than in farmers' fields (Fig. 4A). The presence of *C. plutellae* and the timely application of *B. thuringiensis* contained the population of DBM. With 11 parasitoid releases (from 1st to 3rd planting), a considerable increase of cocoons and parasitism were observed in demonstration and farmers' fields (Fig. 4B and C). The peak of parasitism was recorded in the first week of January (42%) and in May (78%). During these months, the application of insecticides ceases because the cabbage are about to be harvested. With the decrease of DBM population in the field the population of cabbage moth and cutworms (*Spodoptera* spp.) increased. This was followed by the low population of DBM in June. Also the plants during this period were stunted and infected with black rot disease. All the cabbage planted in Nagcarlan in July and August 1990, including our demononstration field, were destroyed by these pests and disease.

Constraints in Field Establishment of *C. plutellae*

Although the parasitism from the demonstration and nearby farmers' fields suggest that continuous introduction of *C. plutellae* with supplementary application of microbial insecticides could regulate the population of DBM, the establishment of this parasitoid in the field will be markedly affected by the following:

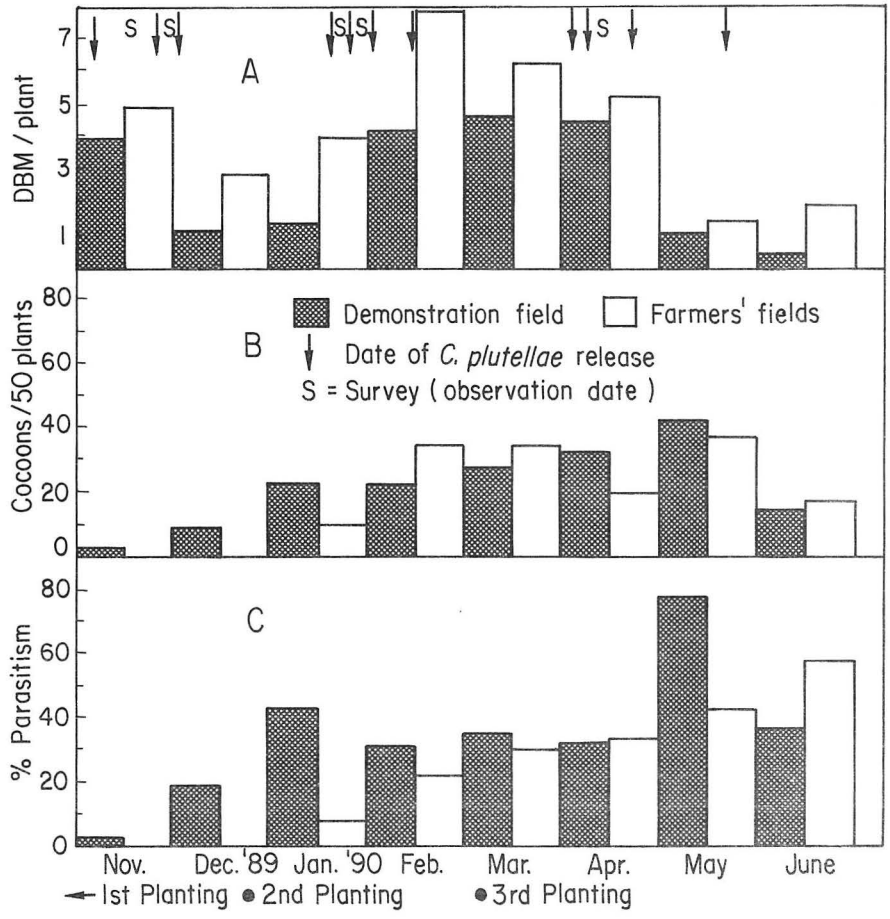

Fig. 4. Population of diamondback moth (A) and *Cotesia plutellae* (B) and parasitism of *C. plutellae* (C) in cabbage at Nagcarlan, Laguna.

1. The propensity of the farmers to use insecticides with knockdown effect, e.g. cartap, rather than slow-acting insecticides and the use of fungicides to control diseases of crucifers. For example copper oxychloride is slightly toxic to *C. plutellae* adults and lowers the parasitization rate (Salazar 1990).

2. The effect of microbial and insect growth regulator sprays in the development of the parasitoid on DBM larvae. These insecticides did not affect the parasitoid adult but considerably reduced adult emergence from the treated host (Salazar 1990).

3. The incidence of parasitic fungus (*Erynia radicans*), presence of hyperparasites (*Trichomalopsis* sp.) and climatic factors that affect the development of the host and the parasitoid.

4. The seasonal planting of cruciferous crops especially in the lowland which caused the disruption of the life cycle of the DBM host.

Recommendations

To hasten the adoption of the technology and the establishment of *C. plutellae* in the field, the following steps are recommended:

1. Set up more demonstration fields with continuous introduction of the parasitoids and conduct training seminars for farmers, emphasizing the importance and methods to preserve the parasitoid in the field.
2. The use of selective insecticides at minimum effective dose to allow the survival of not more than 50% of DBM for parasitization. Studies along this line should be conducted.
3. Develop a low-cost and efficient mass-rearing technique for the host and parasitoid for large-scale production.
4. Breed and select strains of parasitoids resistant to insecticides.
5. Import and search for more potent biocontrol agents to augment *C. plutellae* not only for DBM but also for *C. binotalis* and other cruciferous pests.

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Toxicity of Insecticides to *Cotesia plutellae*, a Parasitoid of Diamondback Moth

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Abstract

Toxicity of 17 commonly used insecticides used to control diamondback moth, *Plutella xylostella* (L.) was evaluated against adults of *Cotesia plutellae* Kurdjumov. The insecticide concentrations studied were equivalent to those recommended in the field. Adults of the parasitoid were released into exposure kits with fresh dry insecticide film for 24 hours, at which time mortalities were noted. The surviving adults were supplied with larvae of diamondback moth for parasitism. Results revealed that carbofuran, cartap, mevinphos, quinalphos, methomyl, methamidophos and deltamethrin were harmful (mortality >99%) to adults of *C. plutellae*, while the remaining 10 insecticides proved to be harmless (mortality < 50%). The effects of insecticides in reducing parasitism of *C. plutellae* in decreasing order were as follows: fenvalerate > acephate > *Bacillus thuringiensis* subsp. *kurstaki* strain NRD-12 (SAN 415) > teflubenzuron > permethrin > chlorfluazuron > acephate > *B. thuringiensis* subsp. *kurstaki* strain HD-1 (Dipel) > fenvalerate > teflubenzuron. Most of these insecticides were considered harmless to parasitism of *C. plutellae* on larvae of diamondback moth except fenvalerate, acephate and *B. thuringiensis* which were classified as slightly harmful. Seven insecticides (quinalphos, deltamethrin, carbofuran, methomyl, cartap, mevinphos, methamidophos) were highly toxic to adults of *C. plutellae* and were used to test their toxicity to the pupal stage of *C. plutellae*. Cocoons at different ages (1-, 2-, 3-, 4-day-old) were sprayed with insecticides at recommended concentrations, and emergence of adults and mortality 24 hours after emergence were recorded. All insecticides tested were harmless to pupae of *C. plutellae*, except quinalphos which was ranked slightly harmful. These results provide further information on the selectivity of insecticides in integrated management of diamondback moth.

Introduction

The diamondback moth (DBM) *Plutella xylostella* (L.), (Lepidoptera: Yponomeutidae) is one of the most important pests of cruciferous vegetables in Taiwan. It has several natural enemies (Wu 1968), and inundative releases of the larval parasitoid *Cotesia plutellae* Kurdjumov apparently exert an effective check of this pest (Chiu et al. 1974). However, it appears unlikely that *C. plutellae* alone could achieve the desired level of control of DBM (Chua and Ooi 1986). Regular insecticide spray to control DBM therefore seems inevitable. However, there is little information available on the toxicity to *C. plutellae* of insecticides commonly used in cruciferous vegetable cultivation (Fan and Ho 1971; Chang 1974; Feng and Wang 1984; Mani and Krishnamoorthy 1984). This may cause serious damage to *C. plutellae* if insecticides applied are not safe for this parasitoid.

It will be possible to practice integrated control measures against DBM only if selective insecticides are chosen. Therefore, investigations were undertaken to develop a standard testing

method to determine the side effects of insecticides on *C. plutellae* and to evaluate toxicity of insecticides against adults and pupae of this parasite with the objective of finding selective insecticides for this parsitoid.

Materials and Methods

Exposure of parasitoids to insecticide was carried out in cages developed by Hassan et al. (1985). The cage consists of an aluminum frame (13 cm long × 13 cm wide × 1.5 cm high) with two square glass plates (13 cm × 13 cm) as ceiling and floor. The walls have ventilation holes coated with black cloth and a slit opening to introduce the parasitoids.

The insecticide treatment was conducted with a Potter Spray Tower (Burkard Co.). Seventeen insecticides were applied at recommended concentrations (Table 1) on the inner surfaces of two glass plates (0.012 ml/cm^2 surface). Water-treated glass plates were used as a control. All tests were replicated five times.

Cotesia plutellae adults were reared on DBM larvae, which in turn were reared on rape seedlings using the method developed by Liu and Sun (1984). Adults (10 pairs, 1-2 days old) were introduced into the exposure cage lined with fresh, dry insecticide film (30 min after spray) and supplied with honey-agar for food. Determination of mortality was made 24 hours after initial exposure. The average number of dead parasitoids was expressed as a percentage of the number released at the beginning of the experiment. The results were compared with the control. The surviving adults were transferred to a screened parasitism cylinder cage (height 15 cm, diameter 21 cm) and offered honey-agar for food and DBM larvae for oviposition. One hundred 2nd-instar larvae of DBM were offered on the 2nd, 4th, 6th and 8th day after treatment. The number of DBM larvae parasitized was calculated for each cage. The reduction in parasitism is expressed as percent of untreated control (= 100%).

Table 1. List of insecticides tested.

Common name	Commercial name	Source	Formulation	Recommended concentration (ppm)	(% a.i.)
deltamethrin	Decis	Teh-Hua	2.8% E.C.	28	0.0028
permethrin	Kestrel	Te-Cheng	10 % E.C.	50	0.005
fenvalerate	Sumicidin	Ruey-Feng	20% W.P.	40	0.004
	Sumicidin	Ruey-Feng	10% E.C.	33.3	0.00333
mevinphos	Phosdrin	Shinung	25.3% E.C.	506	0.0506
methamidophos	Tamaron	VETERANS[a]	50% E.C.	416.7	0.04167
quinalphos	Bayrusil	VETERANS[a]	25% E.C.	500	0.05
acephate	Orthene	Great-Victory	75% S.P.	500	0.05
	Orthene	Great-Victory	25% E.C.	312.5	0.03125
carbofuran	Furadan	Shinung	40.64% F.P.	339	0.0339
methomyl	Lannate	Shinung	24% S.	480	0.048
cartap	Padan	Lih-Nung	50% S.P.	500	0.05
teflubenzuron	Nomolt	BASF	13.57% F.P.	33.9	0.00339
	Diaract	San-Lee	5% E.C.	25	0.0025
chlorfluazuron	Atabron	TPFAACP[b]	5% E.C.	10	0.001
Bacillus thuringiensis subsp. *kurstaki* strain NRD-12	SAN-415	Agro Chem.	8000 IU/mg ISC	5.33 IU/mg	
Bacillus thuringiensis subsp. *kurstaki* strain HD-1	Dipel	Nung-Tai	16000 IU/mg W.P.	16 IU/mg	

[a]VETERANS Chemicals, VACRS., R.O.C. [b]Taiwan Provincial Farmer's Association Agricultural Chemical Plant.

All the testing kits and insects were maintained during the observation period at 25°C, 80% RH and at a photoperiod of 12L:12D in a walk-in growth chamber. The effect of the insecticides on *C. plutellae* was classified by reference to the categories proposed by IOBC/WPRS Working Group (Hassan et al. 1985). Data were statistically analyzed by applying analysis of variance (ANOVA) and Duncan's multiple range test (DMRT).

Seven insecticides which were harmful to *C. plutellae* adults — methomyl, deltamethrin, methamidophos, cartap, carbofuran, mevinphos, quinalphos — were prepared as aqueous solution-based on recommended concentration. Twenty cocoons (1-, 2-, 3-, and 4-day-old) were spread uniformly in a petri dish and sprayed with a Potter Spray Tower with 2 ml of insecticides emulsion. The cocoons were air-dried and kept individually in 24-well culture-plates and placed in chambers at 25°C, 80% RH and 12L:12D. Honey-agar was provided as food. The lid of the culture-plate was punched with needles for ventilation. Cocoons treated with water served as controls. Each treatment was replicated five times. Adults emergence and dead adults 24 hours after emergence were recorded daily. The data were converted to percent mortality and corrected with Abbott's formula. Data were statistically analyzed by applying ANOVA and DMRT.

Results and Discussion

Mortality of *C. plutellae* as a result of exposure to different insecticides is presented in Table 2. Carbofuran cartap, mevinphos and quinalphos caused 100% mortality. Methomyl, methamidophos and deltamethrin caused the second highest mortality of 99.8%, 99.8% and 99.6% respectively. Fenvalerate (40 ppm) caused 30.1% mortality, whereas the remaining insecticides resulted in less than 7% mortality. Among them, Diaract E.C. (teflubenzuron) (25 ppm) and fenvalerate (33.3 ppm) caused no mortality. There were no significant differences between sexes on mortality with all insecticides tested.

Based on the criteria suggested by Hassan et al. (1985) to evaluate the toxicity of insecticides to natural enemies, carbofuran, cartap, mevinphos, quinalphos, methomyl, methamidophos and deltamethrin were rated as harmful. The remaining insecticides, fenvalerate (40 ppm), acephate (500 ppm), teflubenzuron (Nomolt F.P., 33.9 ppm), *B. thuringiensis* subsp. *kurstaki* strain

Table 2. Effect of insecticides on adults of *C. plutellae* 24 hours after exposure.

Insecticides	Mortality (%)[a]	Evaluation[b]
Furadan (carbofuran)	100 a	4
Padan (cartap)	100 a	4
Phosdrin (mevinphos)	100 a	4
Bayrusil (quinalphos)	100 a	4
Lannate (methomyl)	99.80 a	4
Tamaron (methamidophos)	99.80 a	4
Decis (deltamethrin)	99.58%	4
Sumicidin (fenvalerate)	30.12 b	1
Orthene (acephate)	6.49 c	1
Nomolt (teflubenzuron)	4.55 cd	1
SAN 415 (*B. thuringiensis* subsp. *kurstaki* strain NRD-12)	2.85 cde	1
Atabron (chlorfluazuron)	1.84 cdef	1
Kestrel (permethrin)	0.81 def	1
Dipel (*B. thuringiensis* subsp. *kurstaki* strain HD-1)	0.20 ef	1
Orthene (acephate)	0.20 ef	1
Sumicidin (fenvalerate)	0 f	1
Diaract (teflubenzuron + fluvalinate)	0 f	1

[a]Data were transformed to sin-1$\sqrt{x}$ prior to statistical analysis. Values in the column followed by the same letter are not significantly different at 5% level by DMRT. [b]1 = harmless (<50%), 2 = slightly harmful (50-79%), 3 = moderately harmful (80-99%), 4 = harmful (>99% mortality). Hassan et al. (1985).

NRD-12 (SAN 415 ISC, 5.33 IU/mg), chlorfluazuron (10 ppm), permethrin (50 ppm), *B. thuringiensis* subsp. *kustaki* strain HD-1 (Dipel W.P., 16 IU/mg), acephate (312.5 ppm), fenvalerate (33.3 ppm) and teflubenzuron + fluvalinate (Diaract E.C., 25 ppm) were considered as harmless to adults of *C. plutellae*.

The mortality of *C. plutellae* adults caused by quinalphos is consistent with results reported by Mani and Krishnamoorthy (1984), and Chang (1974). They indicated that adults of *C. plutellae* were highly susceptible to quinalphos. Peter and David (1988) reported that quinalphos caused 100% mortality to *Apanteles taragamae* adults. Our results showed that methomyl and mevinphos had adverse effect on *C. plutellae* adults and are in accord with those of Chang (1974) who found the above-mentioned insecticides to be highly toxic to *C. plutellae*. Our results showed that *C. plutellae* was extremely susceptible to cartap, carbofuran and methamidophos. It is not suitable to use cartap, carbofuran, methamidophos, deltamethrin, methomyl, mevinphos, and quinalphos in IPM involving mass release of *C. plutellae* adults, although observations made by Chang (1974) showed that tertiary amines cartap caused only 60% mortality to *C. plutellae* adults.

The pyrethroids were harmless to adults of *C. plutellae*. This agrees with earlier observations on *Apanteles* sp. (Waddill 1978), *A. marginiventris* (Wilkinson et al. 1979), *C. plutellae* (Mani and Krishnamoorthy 1984; Feng and Wang 1984), and *A. taragamae* (Peter and David 1988). However, deltamethrin (Decis E.C., 28 ppm), which caused 100% adult mortality in our study, is an exception. Peter and David (1988) demonstrated that deltamethrin (Decis 2 ppm) killed only 14% of *A. taragamae* adults. Mani and Krishnamoorthy (1984) showed that deltamethrin (Decis E.C., 1.4 ppm) caused 10% mortality of *C. plutellae* adults. The higher dose of deltamethrin used in our study probably caused the higher mortality. A reduced dosage of deltamethrin may have greater potential for use as a selective insecticide in cruciferous ecosystems.

Acephate was one of the least toxic conventional insecticides tested. Our results agree with the previous study of Feng and Wang (1984). Insect growth regulators (IGRs) including teflubenzuron (Diaract), chlorfluazuron (Atabron) and teflubenzuron (Nomolt) are safe for adults of *C. plutellae*. Our studies also indicated that *B. thuringiensis* subsp. *kurstaki* strain HD-1 (Dipel, SAN 415 ISC) had no effect on adults of *C. plutellae*.

The effect of insecticides on parasitism by surviving *C. plutellae* at different time intervals following treatment is shown in Fig. 1. Generally 4 days after treatment the parasitism was highest, followed by 2, 6 and 8 days after treatment. Substantial differences in parasitism were recorded in comparison with the check within each time interval. Diaract showed the highest and fenvalerate (Sumicidin W.P., 40 ppm) showed the lowest parasitism. There was no significant difference in parasitism between the control and Diaract exposure at the 2nd, 4th and 8th days.

Results of the reduction in parasitism by insecticides are shown in Table 3. Based on the criteria proposed by Hassan et al. (1985), most of the insecticides were harmless to *C. plutellae*. Only three out of 10 insecticides were classified as slightly harmful. They were fenvalerate (Sumicidin W.P. 40 ppm, 2 and 8 days after treatment), acephate (Orthene S.P., 500 ppm, 2 days after treatment) and *B. thuringiensis* subsp. *kurstaki* strain NRD-12 (SAN 415 ISC, 6 days after treatment). The effects of the insecticides in reducing parasitism of *C. plutellae* in decreasing order were as follows: fenvalerate (Sumicidin W.P, 40 ppm) > acephate (Orthene S.P., 500 ppm) > *B. thuringiensis* subsp. *kurstaki* strain NRD-12 (SAN 415 ISC, 5.33 IU/mg) > teflubenzuron (Nomolt F.P., 33.9 ppm) > permethrin (Kestrel E.C., 50 ppm) > chlorfluazuron (Atabron E.C., 10 ppm) > acephate (Orthene E.C., 312.5 ppm) > *B. thuringiensis* subsp. *kurstaki* strain HD-1 (Dipel W.P , 16 IU/mg) > fenvalerate (Sumicidin E.C., 33.3 ppm) > teflubenzuron (Diaract E.C., 25 ppm). Concentrations and formulations of IGRs, pyrethroids and *B. thuringiensis* subsp. *kurstaki* appear to have a significant impact in the reduction of parasitism.

The effects of different insecticides on pupae are expressed in percent emergence (Fig. 2). The results revealed that there were significant differences between the seven insecticidal treatments with respect to percentage adult emergence.The maximum toxicity of insecticide to 1-day-old pupae was that of quinalphos followed by mevinphos, carbofuran, cartap, methamidophos, deltamethrin, and methomyl. With few exceptions (Fig. 2), the toxicity of

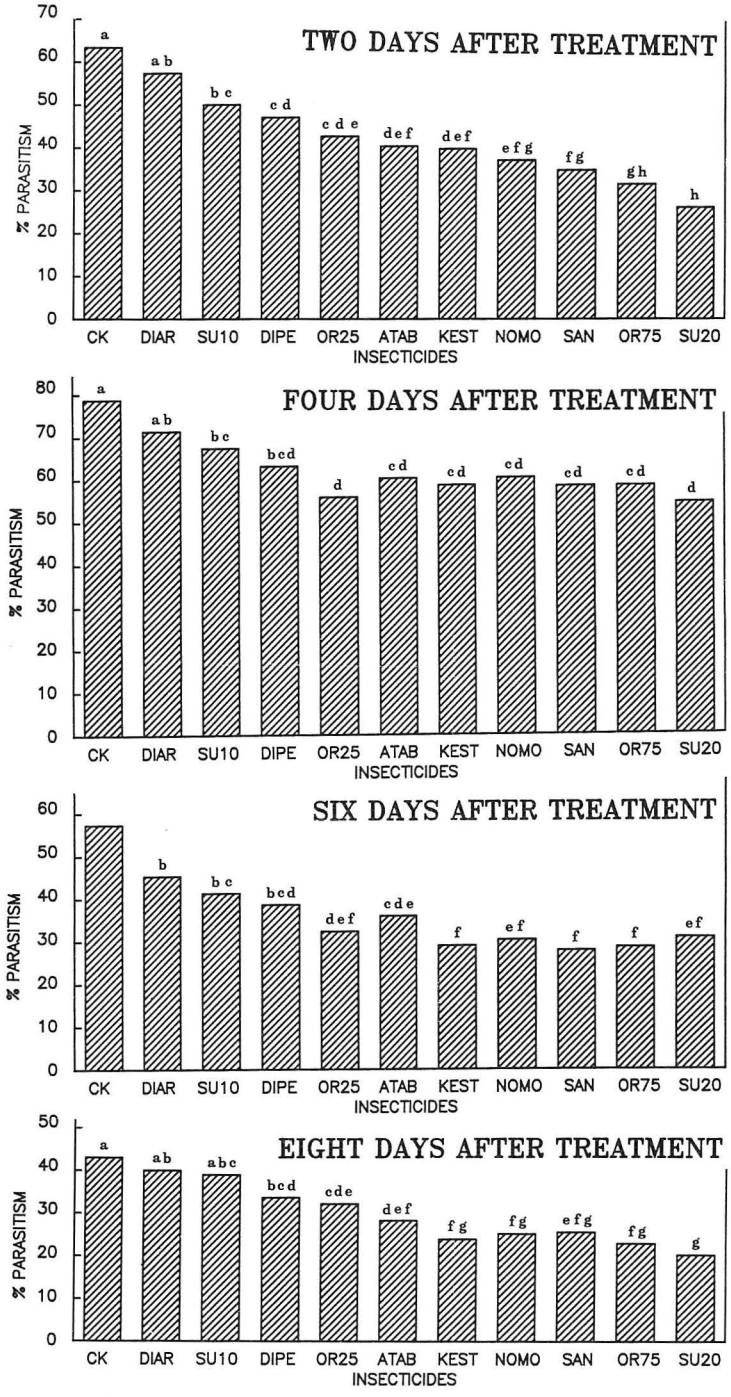

Fig. 1. Effect of insecticides on parasitism of *C. plutellae*. DIAR = Diract; SU10 = Sumicidin 10% E.C.; DIPE = Dipel; OR25 = Orthene 25% E.C.; ATAB = Atabron; KEST = Kestrel; NOMO = Nomolt; SAN = SAN415; OR75 = Orthene 75% S.P.; SU20 = Sumicidin 20% W.P. The values in bars with dissimilar letters significantly differ at 5% level according to Duncan's multiple range test.

Table 3. Reduction in parasitism by insecticides.

Insecticides recommended (concentration)	Reduction (%) after treatment (days)[a]			
	2	4	6	8
20% Sumicidin W.P. (40 ppm)	58.7 a	30.9 a	45.7 ab	52.7 a
75% Orthene S.P. (500 ppm)	50.7 ab	25.5 ab	49.7 ab	46.7 a
8000 IU/mg SAN 415 ISC (5.33 IU/mg)	45.0 abc	24.5 ab	51.0 a	40.6 a
13.57% Nomolt F.P. (33.9 ppm)	41.3 bc	22.8 ab	46.7 ab	40.9 a
10% Kestrel E.C. (50 ppm)	36.6 bcd	22.2 ab	48.3 ab	44.0 a
5% Atabron E.C. (10 ppm)	36.1 bcd	22.7 ab	36.5 bcd	34.2 ab
25% Orthene E.C. (312.5 ppm)	31.6 cde	25.6 ab	42.6 abc	22.7 b
16000 IU/mg Dipel W.P. (16 IU/mg)	25.0 de	15.3 bc	30.8 cd	21.4 bc
10% Sumicidin E.C. (33.3 ppm)	18.9 e	10.3 cd	26.4 de	8.8 cd
5% Diaract E.C. (25 pm)	8.0 f	4.9 d	19.4 e	6.9 d

[a]Data were transformed to $\sin^{-1}\sqrt{x}$ prior to statistical analysis, and the values in each column followed by the same letter were not significantly different at 5% level by DMRT.

inscecticides to 2-, 3-, and 4-day-old pupae exhibited similar trends as to the 1-day-old pupae.

The comparison of percent emergence from 1- to 4-day-old pupae treated with each insecticide is reported in Fig. 2. Results showed that 3- and 4-day-old pupae were more tolerant to methamidophos, cartap and carbofuran than 1- and 2-day-old pupae. However, there were no sigificant differences of percentage emergence between four pupal ages in the other treatments.

The system for grading the toxicity of insecticides to beneficials suggested by Hassan et al. (1985) was also adopted to evaluate the toxicity to pupae in our study (Table 4). Quinalphos, which caused 54.9% mortality to 4-day-old pupae, was rated as slightly harmful. The remaining insecticides were considered harmless to pupae of *C. plutellae*.

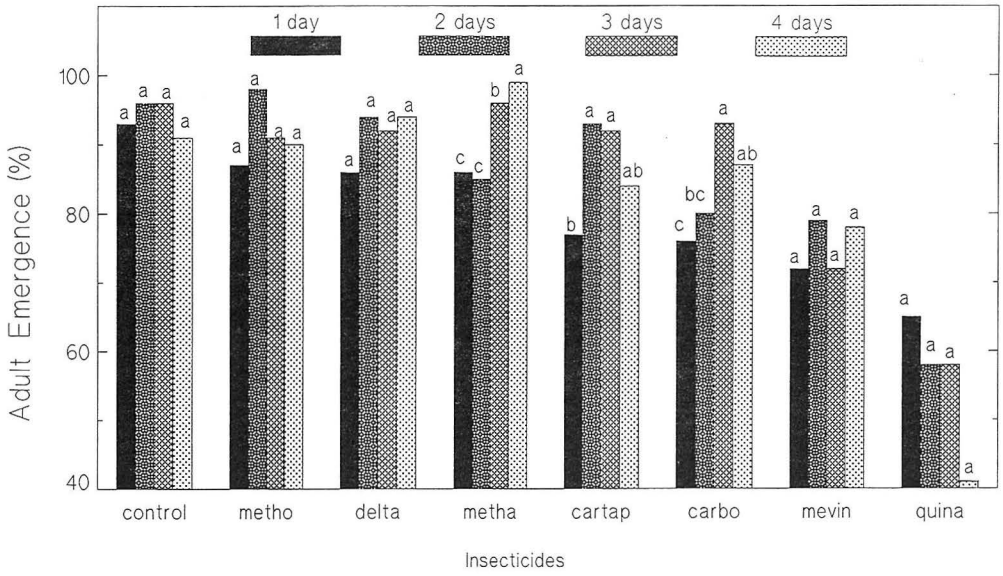

Fig. 2. Effect of various insecticides applied to 1-4 day-old *C. plutellae* pupae on the emergence of adults. The bars with dissimilar letter are signifiantly different at 5% level according to Duncan's multiple range test.

Table 4. The effects of insecticides on pupae of *C. plutellae*.

Insecticides	Mortality (%)[a] and toxicity rating[b] at different pupal ages							
	1 day		2 days		3 days		4 days	
	Mort.	Rating	Mort.	Rating	Mort.	Rating	Mort.	Rating
Lannate (methomyl)	6.5	1	0	1	5.2	1	1.1	1
Decis (deltamethrin)	7.5	1	2.1	1	4.2	1	0	1
Tamaron (methamidophos)	7.5	1	11.5	1	0	1	0	1
Padan (cartap)	17.2	1	3.1	1	4.2	1	7.7	1
Furadan (carbofuran)	18.3	1	16.7	1	3.1	1	4.4	1
Phosdrin (mevinphos)	22.6	1	17.7	1	25.0	1	14.3	1
Bayrusil (quinalphos)	30.1	1	39.6	1	39.6	1	54.9	2

[a]Means of five replications. Mortality is expressed in percent of control. [b]1 = harmless (<50%), 2 = slightly harmful (50-79%), 3 = moderately harmful (80-99%), 4 = harmful (>99% mortality). Hassan et al. (1985).

Mortality of newly emerged adults (1-day-old) from insecticide-treated pupae is shown in Fig. 3. Mortality with quinalphos and mevinphos was the highest. The remaining treatments

caused <5% mortality. Mortality of newly emerged adults from 2-day-old pupae treated with mevinphos was the highest (10%). However, mortality of newly emerged adults from 3- and 4-day-old pupae treated with insecticides was very low.

A comparison of mortality of newly emerged adults (1-day-old) between four pupal ages affected by each insecticide is shown in Fig. 3. There was a general tendency for the mortality to decrease as the pupae grew older. Once the adults emerged from the insecticide-treated pupae, the chances of being affected by the insecticide residue from cocoon within 24 hours seemed relatively small.

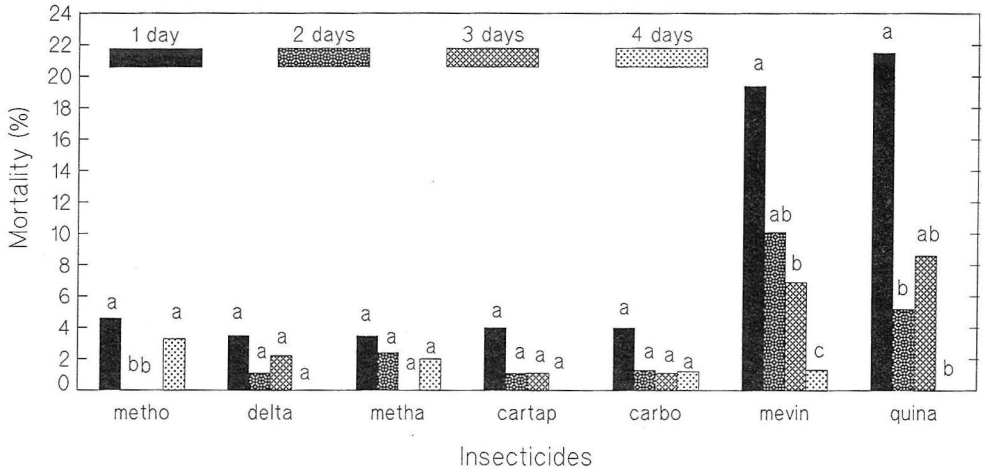

Fig. 3. Mortality of newly emerged *C. plutellae* adults, the pupae of which were treated with various insecticides when they were 1-4-day-old. Values in bars with dissimilar letters are significantly different at 5% level according to Duncan's multiple range test.

Conclusions

Adults were susceptible to insecticidal treatment but the robust life stage-the pupal stage - was relatively tolerant. This agrees with earlier observations on *C. plutellae* (Fan and Ho 1971; Mani and Krishnamoorthy 1984). Judging from direct contact toxicity to pupae and residual toxicity from treated cocoons, with the exception of quinalphos, the insecticides tested harmful to adults but harmless to all pupal stages of *C. plutellae*.

Although the insecticides were applied in recommended concentrations, the test methods must nevertheless be regarded as more stringent despite the optimal conditions under which the natural enemies were kept (feeding, ventilated cages). Compared with the insects kept in the glass cage with its contaminated surface, organisms in the field have a chance to survive on noncontaminated parts of the plants. Therefore, it can be concluded with a high degree of certainty that insecticides which proved to be harmless in the laboratory tests are also harmless in the field (Hassan 1983).

IPM will be most effective if the insecticides are effective against pests and relatively safe for beneficials. Insecticides with these desirable attributes go well with biological control, and will reduce the side effects of insecticide usage. The variations in the response of the parasitoids to test insecticides may help in management of DBM. Selection of the right insecticide and proper timing of application would also be useful tools, both in conserving beneficials and achieving good control (Lingren et al. 1972). We suggest rotational application both in time and/or space, of selective insecticide, i.e. teflubenzuron (IGR), fenvalerate (pyrethroid), *B. thuringiensis* subsp. *kurstaki* and acephate (OP) in combination with inundative release of *C. plutellae* adults to achieve

sound IPM of DBM in cruciferous ecosystems. Application of commonly used insecticides (except quinalphos), though harmful to adults of *C. plutellae*, could be timed to coincide with the occurrence of high population densities of cocoons which are less affected by insecticides. These results further selectivity in IPM for DBM.

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Inundative Release of *Trichogramma* for the Control of Cruciferous Lepidoptera: Preintroductory Selection of an Effective Parasitoid

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Abstract

Egg parasitoids of the genus *Trichogramma* are the most widely used natural enemy for biological pest control in the world. Their use is restricted to lepidopterous pest species. Due to relatively cheap mass rearing systems employing factitious host species, *Trichogramma* are usually applied for inundative biological control: repeated releases of high numbers of parasitoids aiming at a direct control effect in the pest population. This paper discusses one of the central issues of biological control: the selection of a candidate natural enemy. Studies of the parasitization and searching behavior of *Trichogramma* have shown that inter- and intra-specific variability occurs for most characteristics determining their effectiveness as a control agent. A preintroduction selection procedure comparing different *Trichogramma* strains or species may then be useful or even necessary. This procedure requires a set of evaluation criteria and bioassays to quantify these.

Inundative Biological Control

Inundative biological control aims at directly increasing mortality in the pest population, whereby the released natural enemy is used as a biological insecticide (Stinner 1977). The practice of inundative release therefore centers on its cost/benefit ratio as compared to alternatives, especially pesticides. Species of the hymenopterous genus *Trichogramma* have been used more than any other natural enemy for inundative biological control (Ridgway and Morrison 1985). *Trichogramma* has a wide range of hosts, especially among the Lepidoptera (Nagarkatti and Nagaraja 1977). Suitable factitious hosts include the eggs of lepidopterous storage pests of grains, making mass production economically feasible.

Experiments with *Trichogramma* started in the beginning of this century in the USA and the USSR. Development of a method for mass production of *Trichogramma* on eggs of the Angoumois grain moth *Sitotroga cerealella* gave rise to worldwide use of the parasitoid as a control agent. In a paper with the meaningful title 'Is *Trichogramma* becoming a fad?', Smith and Flanders (1931) expressed their concern about the usefulness of these applications, on the grounds of a lack of convincing evidence for practical results. Because of this, and the advent of organic chemical pesticides, interest in *Trichogramma* faded in western countries (Ridgway et al. 1977). Research on the use of *Trichogramma* continued in the USSR and China, probably due to a lack of pesticides, rather than biological or economical effectiveness of *Trichogramma* release (Van Lenteren 1987). In these two countries application is presently carried out on several millions of hectares.

Increasing problems with the use of pesticides has stimulated renewed interest in biological control, whether as a method by itself or as part of an integrated pest control system. For

Trichogramma, this has led to commercially successful programs in Europe to control the corn borer, *Ostrinia nubilalis*, by inundative releases of *T. maidis* Pintureau and Voegelé (Hassan 1981, Bigler 1986). Prospective application of *Trichogramma* against numerous other lepidopterous pests throughout the world has been reviewed by Stinner (1977). Successful applications are reported for some crops, while results appear to be inconsistent for others. For example, extensive research into the control of *Heliothis zea* in cotton by releases of *T. pretiosum* Riley did not provide sufficient results to warrant general adoption of this method in the USA (King et al. 1986). Effective control of Lepidoptera on cabbage by releases of *T. evanescens* Westwood has been reported for the species *Mamestra brassicae* (Shchepetilnikova 1974, Hassan and Rost 1985), *Pieris rapae* (Parker et al. 1971, Parker and Pinnell 1972) and *Trichoplusia ni* (Zilberg 1972). Parasitism of *Plutella xylostella* (L.) eggs is scarcely reported in the literature. In Japan they are parasitized by *T. chilonis* Ishii (K. Hirai, personal communication).

Egg parasitoids account for 15% of the successful inoculative biological control agents, and their success ratio is similar to that of larval parasitoids and better than that of pupal parasitoids (Hokkanen 1985). Egg parasitoids occur in 16 families of Hymenoptera, of which the Trichogrammatidae, Mymaridae and Scelionidae are composed exclusively of egg parasitoids. Examples of successful inoculative control by egg parasitoids are known for the Scelionidae and the Mymaridae, but not for the Trichogrammatidae. Greathead (1986) therefore concluded that trichogrammatids are less successful control agents than species from the other egg-parasitoid families. Strand (1986) suggested that, for unknown reasons, *Trichogramma* spp. are less specialized in parasitizing certain species within their major host group (Lepidoptera) than the scelionids (specialized on Lepidoptera, Hemiptera of Orthoptera) and the mymarids (specialized on Homoptera). The preoccupation with *Trichogramma*, instead of other egg parasitoid, for inundative biological control seems to stem largely from its polyphagous nature and feasibility of mass production on factitious hosts, rather than from superior biological traits determining effectiveness as a control agent.

Selection Of Candidate Natural Enemies

The major phases in the development of a biological control program are: (1) collection of information on pest and natural enemies, (2) exploration for natural enemies, (3) selection of candidates, (4) mass production and release, and (5) evaluation of results.

The selection of candidate natural enemies, which is generally considered the most critical phase for the success of a biological control program, is an issue surrounded by conflicting ideas about the usefulness of preintroductory research. This 'art versus science' conflict (Harris 1973) seems to be one between theoretical ecologists and practitioners of biological control. In the extreme, the theoreticians believe that it is possible to determine the 'best' natural enemy by preintroductory studies, whereas the practitioners advocate a minimum of such studies and prefer to test each prospective candidate directly in the field by a trial-and-error method. Nevertheless, most practitioners express ideas about attributes that an effective natural enemy should possess, although they rarely seem to use these as a check list of selection criteria (Van Lenteren 1980).

Selection Of *Trichogramma*

The feasibility of mass production on factitious hosts has promoted extensive use of the egg parasitoid *Trichogramma* for inundative biological control programs. In production systems the hosts used are generally grain-feeding Lepidoptera such as the rice meal moth, *Corcyra cephalonica*, the Mediterranean flour moth, *Ephestia kuehniella*, and the Angoumois grain moth, *Sitotroga cerealella*. In addition to efficient mass production, application systems have been developed for manual delivery in small fields of 1-2 ha and aerial delivery in fields larger than 10 ha.

Scholz (1990) reviewed the different selection methods employed by *Trichogramma* workers from literature data and concluded that preintroduction evaluation studies of different candidates are carried out in less than 10% of all programs. The prospective *Trichogramma* strain is most often collected locally, on the grounds of expecting an optimal adaptation to pest and environment in local populations, or is imported from a colleague who uses it successfully against the same pest species (Table 1).

Selecting *Trichogramma* by means of trial-and-error releases in the field has produced good results, but not consistently (Stinner 1977). The selection of effective parasitoid species or strains has therefore been identified as one of the necessities to improve the success record for *Trichogramma* application (Ridgway and Morrison 1985).

Table 1. Criteria used to select species of egg parasitoids for use against lepidopteran pests around the world.

Method of selection	Frequency (%)
Pre-release evaluation	8.6
Species reared from target pest	25.7
Most abundant species in release region	31.4
Species readily available from a research institution or biological control company	20.0
Species successfully used in other countries	5.7
Method of selection not specified	8.6

Inter- and intra-specific variation

In preintroductory selection programs of candidate natural enemies researchers make use, among others, of the fact that pest species usually have a different complex of natural enemies for different parts of their natural range. Biological differences among species or populations of natural enemies, whether morphological, physiological, ecological or behavioral, are the result of geographical variations in environmental and biological interactions and reflect adaptations in populations to the local environment. Little is known, however, about qualitative and quantitative genetic differences between conspecific populations of natural enemies which differ in biological traits.

In the past, researchers have shown a certain awareness of the importance of genetic variation for the success of (inoculative) introductions of natural enemies (Remington 1968; Hoy 1985). A prolific use of different terms in the literature to characterize different populations of conspecific natural enemies, including form, race, ecotype, biotype, strain and polymorphs, bears witness of a similar awareness among practitioners of biological control (Diehl and Bush 1984).

Introduction of differently adapted strains or species may improve the success of a biological control program. Messenger and Van den Bosch (1971) reviewed several examples of limited adaptiveness in geographical strains of natural enemy species, such as climatic tolerance, host specificity and evasion of host-immunity response, which reduced their effectiveness as control agents. Nevertheless, they concluded that this vast potential resource of conspecific strains of natural enemies with different ecological and behavioral adaptabilities has hardly been tapped to improve biological control.

A preintroductory selection program for *Trichogramma* will make use of inter- and intra-specific differences between different populations of the genus. In the past, only a few species of *Trichogramma* were recognized, due to taxonomic difficulties (Nagarkatti and Nagaraja 1977). Differences in structure of male genitalia were used to describe new species by Nagaraja and Nagarkatti (1969) and gave rise to extensive revision of the genus (Voegelé and Pintureau 1982). Therefore, it is difficult to relate previous accounts of biological differences between ecotypes of *Trichogramma* (Kot 1979), or strains (Diehl and Bush 1984), with their present taxonomic status. Inter- and/or intra-specific differences have been reported among *Trichogramma* for

various biological characteristics (see Pak (1988) for references): (1) adaptation to plant structure or habitat, (2) adaptation to climatic conditions, (3) fecundity, (4) longevity, (5) intrinsic rate of increase, (6) host suitability, (7) walking speed, (8) parasitization rate, (9) attraction to hosts, and (10) response to searching stimulants (kairomones).

Selection Criteria

Consideration of current knowledge and ideas on factors determining the effectiveness of natural enemies in suppressing pest populations has led to a list of nine possible criteria for preintroductory selection of natural enemies (Van Lenteren 1986). These selection criteria are discussed below. Definitions of most of these criteria are reviewed and, as a consequence, renamed to clarify their meaning. Conclusions are summarized in Table 2, in which each criterion is given a coarse rating (low-medium-high) for three aspects; (1) usefulness to select candidate strains, (2) the amenability for manipulation by, for example, producers and users, and (3) the current level of biological understanding on the way certain traits function and may be utilized. In addition, important research areas are indicated for each criterion.

Table 2. Evaluation of criteria for the selection of candidate *Trichogramma* strains to be used in inundative biological control programs and major research areas identified to enhance the evaluation of selection criteria.

Criterion	Usefulness for preintroductory selection	Amenability for manipulation by applier	Level of knowledge or utilization	Research areas
Environmental risks	high[a]	medium	low	Impact of releases on natural host communities
Tolerance of climatic extreme	high	medium	medium	Heritability of locomotory activity
Host-plant adaptation	medium	low	low	Dispersal to different habitats or plant types
Host selection	high	low	medium	Host selection in natural patches; host perception
Host suitabilty	medium	low	medium	Cytolytic process of killing hosts
Seasonal synchronization	low	high	high	Longevity of wasps in the field
Reproductive capacity	medium	high	high	Heritability of fecundity
Host-finding capacity	high	medium	low	Travel speed, dispersal by flight, role of kairomones
Culture method	high[a]	high[b]	medium	In vitro culture, quality of produced wasps

[a]Conditional necessity rather than selection criterion for research program. [b]Manipulation by producer.

Environmental risks

Serious negative aspects of natural enemies, such as (facultative) hyperparasitic habits of parasitic wasps or predation upon other beneficial species, may impede their use for any method of biological control. However, the primary concern is not with the negative trait itself, but rather with the degree of risk it may pose to the environment. Environmental risk therefore seems to be a more suitable term for this criterion.

Inundative biological control involves repeated releases of large numbers of natural enemies into crops. The possible impact of releases on alternative hosts in surrounding (natural) habitats

should therefore be investigated as part of the development of the control program. Registration of biological control agents, comparable to that of pesticides, will probably become a regulation, which will require criteria to test their environmental safety. *Trichogramma* inundations are considered safe by appliers, perhaps because they usually release local strains collected from the pest species. However, if an exotic strain is released, or one that has been artificially selected, the possibility of increased egg parasitism of Lepidoptera other than the pest species itself is not imaginary, especially since most *Trichogramma* species are polyphagous. Permanent establishment of exotic species might be avoided by selecting strains which, for example, cannot overwinter in the new environment.

Tolerance of climatic extremes

Climatic adaptation involves the ability of natural enemies to effectively control a pest under ambient climatic conditions. However, in a broader sense, climatic adaptation concerns the ability of natural enemies to tolerate the extreme abiotic conditions of their environment. Climatic tolerance is a determining factor for the survival and/or the reproduction of a natural enemy. For inoculative introductions, survival under extreme conditions is an essential prerequisite for colonization, even if reproduction is limited to periods of more favorable conditions. For seasonal inoculative and inundative introductions the ability to reproduce is of immediate importance and should not be restricted by abiotic conditions.

In *Trichogramma*, tolerance to extreme environmental conditions has been primarily studied for high temperatures. However, in countries with a temperate climate, low temperatures may be a major limiting factor to the parasitization activity of *Trichogramma* females (see host-finding capacity). Significant variations in proportional activity (i.e. the number of females engaged in locomotory and parasitization activity) at 12 °C were found by Pak and Van Heiningen (1985) between geographical strains and appeared to correspond to the performance of strains of different activity released in the field (Pak et al. 1989). Temperature dependence of parasitoid activity, therefore, appears to be a useful criterion to select candidate strains for evaluation programs.

Host-plant adaptation

The ability to attack a given species of host on all economically important host plants is considered important for inoculative biological control, but not for seasonal inoculative and inundative biological control. For the latter methods it has been suggested that a variable effectiveness of a natural enemy for different host plants of its (polyphagous) insect host is amenable for manipulative correction, e.g. by releasing more enemies.

Different rates of egg parasitism are reported for the same host species on different plant types, usually by different *Trichogramma* spp. (Martin et al. 1981; Lopez et al. 1982). Host-plant adaptation may be involved in host-habitat location, which has been demonstrated by Altieri et al. (1981) and Nordlund et al. (1985). Furthermore, *Trichogramma* species appear to be adapted to different plant structures (Thorpe 1985). Host-plant adaptations probably interact with host-species adaptations, and their combined effect results in strains of *Trichogramma* being specialized and released in different crops, for example *T. maidis* against the corn borer, *O. nubilalis*, and *T. pretiosum* against the cotton bollworm, *H. zea*.

Host specificity

Host specificity concerns the range of different host ages and host species accepted by the parasitoid. As a selection criterion host specificity is related to other criteria, such as environmental risks (narrow host range and low propensity of hyperparasitism), host suitability and density responsiveness (high host-finding capacity). If the latter criteria are evaluated for candidate parasitoids, host specificity might be investigated separately. Alternatively, a study of host specificity might be sufficient to permit evaluation of the other criteria also.

For inundative and seasonal inoculative releases host specificity is not considered an important selection criterion, apparently due to the assumption that releases are directed against a single pest species (Van Lenteren 1980). However, if two or more related species are to be controlled, the use of a single effective parasitoid is probably more economical (in costs of development of the program) than the use of different parasitoids for each host species. Host specificity, therefore, should be considered as an important selection criterion, because the occurrence of related host species in the same crop is not exceptional (Ehler and Miller 1978).

Pak et al. (1986) and Pak et al. (1990b) studied variability among *Trichogramma* strains for host-age and host-species selection of host species occurring on cabbage crops. The preferred ages of different species of host eggs had to be determined before host-species selection experiments could be carried out. The effectiveness of a parasitoid may be reduced if host acceptance is limited to certain ages of hosts. Host age selection scarcely occurred, but females of 9 out of 12 strains tested for host species selection preferred *Mamestra brassicae* eggs over *Pieris brassicae* and *P. rapae* eggs as a host. Host-species selection turned out te be a useful criterion for the selection of candidate strains, because parasitism of *Pieris* eggs in the field was predominantly observed for a strain that did not show a preference for either of the host species in the laboratory (Pak et al. 1989).

Host suitability

Host suitability concerns the ability of the parasitoid to complete development in the host and seems a more direct term for this criterion than internal synchronization. It was not considered an important selection criterion for inundative releases by Van Lenteren (1980), because their aim may be fulfilled if the host is killed. However, if host eggs are not always killed when attacked (Pak et al. 1990a), host suitability should be considered as a criterion in the selection of candidate strains.

Seasonal synchronization

Seasonal synchronization with the host refers to the temporal (external) correspondence between the proper stages of the life cycles of host and parasitoid. For inundative biological control, natural synchronization is not an important criterion. The applier can synchronize populations of the parasitoid and the host by introducing the parasitoids at the proper time. In fact, inundative releases are a way of artificially synchronizing the occurrence of hosts and sufficient numbers of parasitoids in places where they are actually poorly synchronized. Host phenology, especially host-egg density, determines decisions regarding the timing and frequency of releases. Thus, the presence of host eggs must be regularly monitored or predicted by means of model computations (Ridgway et al. 1981).

The age of host eggs may significantly affect their susceptibility to parasitism by *Trichogramma* (Pak 1986). Since young eggs are generally most susceptible, it may be of crucial importance that female wasps are searching for host eggs from the beginning of the oviposition period of the host insect. *Trichogramma* are usually introduced into the field as immatures in the pupal or pharate adult stage, mechanically or by hand. In the laboratory, wasps fed with honey may live for a few weeks at moderate temperatures, but little is known about the life span of females in the field. Under variable, unpredictable weather conditions the introduction of parasitoid pupae of mixed ages into the field may provide for a continuous supply of sufficient foraging wasps for several weeks (Hassan 1982; Bigler 1986).

Reproductive capacity

The potential reproductive capacity of a natural enemy is usually expressed by the intrinsic rate of natural increase (r_m value) which combines development time and mortality of immatures, adult survival and fecundity into the rate of population growth (Pak and Oatman

1982b). Parasitoids develop to the detriment of their hosts (solitary or gregariously), and may also kill them by host feeding. Thus, the host-death rate caused by activity of the parasitoids should offset the rate of increase of the host in the absence of parasitoids (Van Lenteren 1986). The potential reproductive impact on the host population is usually determined in the laboratory under optimal conditions of host availability. In practice, however, parasitoids may not find the hosts as readily as in the laboratory and, consequently, the field host-death rate per female parasitoid may be lower.

For inundative biological control the reproductive potential does not appear to be a useful selection criterion, because a limited parasitoid fecundity can, in theory, be adjusted by releasing more parasitoids. However, Smith and Hubbes (1986) did find that differences in field performance between various indigenous geographical strains of *T. minutum*, in parasitism of spruce budworm eggs, *Choristoneura fumiferana*, corresponded to differences in reproductive capacity between strains determined in the laboratory.

If host eggs are laid in clusters rather than singly, the effectiveness of a parasitoid as a control agent may be reduced if a single female cannot parasitize all hosts in a patch. Field observations by Pak et al. (1989) showed that the fecundity of *Trichogramma* females may be too low to parasitize each egg in clusters of *M. brasssicae* containing more than 20 eggs, which make up a considerable fraction of the egg population, especially toward the end of the season. Thus if the biological control effectiveness of wasps is limited by their reproductive capacity, fecundity might be a useful selection criterion for inundative biological control.

Host-finding capacity

Density responsiveness is often mentioned as a selection criterion related to the host-finding ability of natural enemies. The question of density-dependent host-parasitoid interactions, such as spatial heterogeneity, parasitoid aggregation and mutual interference, is of great interest in the theory of population regulation by effective natural enemies (Waage and Hassell 1982). As a result, density-dependent behavior is commonly seen as an essential feature of the searching efficiency or capacity of candidate natural enemies, but it also seems to be the most difficult selection criterion for experimental evaluation (Van Lenteren 1986).

Coexistence of pest and natural enemy at a low density seems to be an essential feature of inoculative and seasonal inoculative biological control, but not of inundative biological control. In addition to density-dependent behavioral responses of parasitoids, any trait of their searching behavior that has a significant influence on the probability of host-finding might be a useful selection criterion, especially traits related to the searching effort (travel speed, travel time and responses to searching stimulants). Since the measure of success of a natural enemy is in the actual finding of hosts rather than in the searching for hosts, host-finding capacity seems the most appropriate term for this criterion.

The host-finding capacity of a parasitoid is the number of hosts found per unit of time. However, this does not seem to be a useful measure for evaluation, because it is only conveniently determined under controlled conditions at a relatively high host density (Hassell, 1986). At a high host density, the host-finding capacity of a parasitoid is limited by her egg complement and/or by her handling time. However, in fields with relatively low host densities, and especially when host eggs are laid in clusters, wasps may be foraging a lot on leaves without hosts (Pak et al. 1989).

The host-parasitoid interaction occurring in the field suggests that it is practically unrealistic to express the host-finding capacity of inundatively released *Trichogramma* in terms of an absolute deterministic measure, such as the area traversed or the area of discovery. These measures assume that parasitoids search randomly, whereas it is becoming increasingly apparent that host searching is directed or stimulated by physical or chemical cues from the habitat or host, substances functioning as synomones of kairomones.

Analysis of host-finding differences among *Trichogramma* in the laboratory seems to be feasible. For example, Bigler et al. (1988) found that differences in travel speed between a number

of strains of *T. maidis* in the laboratory corresponded consistently, i.e. for several seasons, to differences in performance in the field. This suggests that travel speed might be a useful measure to select strains. Another possibility to select *Trichogramma* strains might be based on differences in the response of females to kairomones. Contact kairomones are present in the wing scales of adult hosts and are deposited by ovipositing females on and around the eggs (Gross 1981). In addition, the sex pheromone of the adult-female host has been found to function as volatile kairomone for *Trichogramma*, probably playing a role in host community location (Lewis et al. 1982). Since sex pheromone is a more host-specific kairomone source than host scales (Noldus 1989), inter- and intra-specific differences in kairomonal responses among *Trichogramma* strains may be more common for volatile than for contact kairomones.

Culture method

A good culture method is an essential prerequisite for the use of a natural enemy in seasonal inoculative and inundative biological control programs. The ability to culture a given natural enemy is a conditional necessity of concern to the producer, rather than a useful selection criterion for the researcher conducting an evaluation program. The widespread use of *Trichogramma* as an inundatively released control agent is a consequence of the feasibility of mass production on factitious host species. However, host-specific species or strains, such as *T. nubilale*, cannot be readily cultured on factitious host eggs, which may limit their practical use (Burbutis and Goldstein 1983).

Host species commonly used to mass-produce *Trichogramma* have relatively small eggs, which produce small *Trichogramma*. Smaller *Trichogramma* females generally have a lower fecundity, a shorter longevity and a lower walking speed than larger females from natural hosts. In the past, the quality of mass-produced *Trichogramma* has received little attention from practitioners, although several accounts of reduced field effectiveness have been reported for laboratory cultures of strains. Adverse genetic changes that might take place in mass-cultured strains should be monitored in order to maintain the quality of *Trichogramma* as a control agent. Population-genetic studies and the monitoring of the quality of mass-produced parasitoids require the quantification of biological traits (Bigler et al. 1987), for which several of the presently discussed selection criteria may be useful.

Improving *Trichogramma* effectiveness

If an effective 'wild' strain of *Trichogramma* cannot be found in nature, it might be possible to improve the effectiveness of existing laboratory strains. The major approaches suggested toward this aim are enhancement of the activity of releases of parasitoids in the field, and genetic improvement of a strain by selective breeding.

Trichogramma has been the subject of extensive research on manipulation of the searching behavior by the application of contact or volatile kairomonal substances to the habitat, which primarily at low host densities might increase the effectiveness of host finding (Gross 1981). Gross et al. (1981) demonstrated that prerelease oviposition and kairomone experience may stimulate searching behavior and increase retention time of wasps in a target area. This response might suppress dispersal of the wasps from a crop habitat. In order to reinforce the response to contact kairomones applied to the habitat, Gross et al. (1984) distributed supplemental (sterilized) host eggs in the field, which increased the rate of finding natural hosts.

The present research on selection criteria to evaluate the effectiveness of *Trichogramma* strains may be useful in directing which qualitative traits need genetic improvement of the parasitoids, because the lack of criteria to measure quality or to direct linear selections appears to be a major limiting factor for putting ideas into practice (Hoy 1976). If natural selection acts against the artificially selected trait, or if outbreeding with a natural population occurs, an artificially selected enemy will gradually revert to a 'wild' genotype after inoculative introduction. The prospects of genetic improvement seem to be especially promising for inundatively introduced

natural enemies, because this method offers the opportunity to maintain the quality of the selected trait of a strain by applying a continuous selection pressure in the mass production.

Art Or Science

Inundative biological control is a technical enterprise, which offers opportunities to leave behind the primitive 'hunting-and-gathering' phase of biological control (Hoy 1979). The trial-and-error approach to the selection of candidate control agents has been a useful element of this phase.

In the past, several ecologists and entomologists have expressed their concerns about the orthodox approach to the choice of effective natural enemies. Krebs (1972) commented that 'until we can explain why an introduction program is a success or a failure, biological insect pest suppression will remain an art, not a science'. Van Lenteren (1980) discussed the question of whether the art does have to become a science, while Hokkanen (1985) suggested that the selection of effective natural enemies 'should probably best be regarded as an art, based on science'.

I believe that if biological control is to gain wide adoption as a method of pest control, it needs to be based on research by scientists, who should enjoy art to give relaxation to their puzzled and weary minds. These scientists should study behavioral interactions between natural enemies and their hosts or prey in order to find useful criteria and methods to evaluate or monitor the effectiveness of candidate natural enemies.

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Life Table of Diamondback Moth and Its Egg Parasite *Trichogrammatoidea bactrae* in Thailand

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Abstract

The life table study of diamondback moth, *Plutella xylostella* (L.), in a naturally infested plot was carried out in 1985-89 in the highland area of Petchaboon Province (1985-86) and the lowland areas of Nakornprathom Province (1987-88) and Kanchanaburi Province (1988-89). The estimated number of generations of diamondback moth equals 17 and 25.5 per year in the highland and lowland areas, respectively. In highland areas, the survival rates in the hot and dry seasons were higher than that in the wet season. In the lowland areas the survival rate in the dry season was higher than that in wet season. Mortality of diamondback moth is due to rainfall and parasites. The parasites found in the highland area are egg parasite (*Trichogramma confusum*), larval parasite (*Cotesia plutellae* Kurdjumov) and pupal parasite (*Diadromus collaris* Gravenhorst). In the lowland areas, egg parasite (*Trichogrammatoidea bactrae* Nagaraja) was found for the first time attacking diamondback moth eggs in Thailand. Its parasitism was 16.2-45.2%. Larval parasite (*C. plutellae*) was also found in the lowland area parasitizing 6.1-32.4% of diamondback moth larvae. In the 1989-90, laboratory study on the insecticide toxicity to adults of *T. bactrae* indicated that fenvalerate, cycloprothrin, ethofenprop, abamectin, chlorfluazuron and *Bacillus thuringiensis* had low toxicity but phenthoate, fenvalerate, ethofenprox and benfuracarb had high toxicity to diamondback moth eggs parasitized by *T. bactrae*.

Introduction

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), is one of the most serious insect pests of cruciferous crops in Thailand. Farmers normally protect the crops from DBM only by spraying insecticides. The insecticide usage becomes not only useless but also harmful when DBM develops resistance. It is therefore most desirable to establish an integrated pest management (IPM) system which is not completely dependent on synthetic insecticides. The development of a life-table is indispensable for the development of an IPM program. The study of factors contributing to DBM survival both for highland and lowland areas in Thailand began in 1985, leading to the development of a DBM life table. *Trichogrammatoidea bactrae* Nagaraja, one of the important egg parasites, was found for the first time during the life table experiment. The Department of Agriculture in Thailand has been successful in mass rearing *T. bactrae*. Hassan et al. (1988) proposed a four-class harmful index to judge the toxicity

of various insecticides to *Trichogrammatoidea*. According to this index, class one is harmless = mortality < 50%; class two is slightly harmful = mortality 50-79%, class three is moderately harmful = mortality 80-99%, and class four is harmful = mortality > 99%. We used these indices to judge the toxicity of commonly used insecticides in Thailand to *T. bactrae*.

Materials and Methods

Three tentative locations were prepared for the study in naturally DBM infested plots: Khao Khor, Petchaboon Province, was selected as the highland area to carry out the experiment during 1985-86; Nakornprathom and Kanchanaburi provinces were selected as the lowland areas to carry out the experiment in 1987-89. Cabbages were planted every 2 months in an area of about 400 m^2. Twenty-four plants were sampled randomly at each crop growth stage and examined for all stages of DBM at 10-day intervals. For Nakornprathom, the plants were planted in the 300 m^2 field. Thirty plants were sampled randomly every other day. The living larvae were separated into small groups (about 10 individuals) and were kept with a piece of cabbage leaf in a plastic container. Pupae were separated and placed one per vial. The insects were reared at 25°C, and parasitism was recorded. Egg, larval and pupal parasites of DBM were separated and placed individually in vials.

One-day-old adult *T. bactrae* were prepared for the toxicological experiment. A filter paper (1 × 3 cm) was dipped in the insecticide solution for about 10 sec, air-dried, then put into a glass vial (2 × 5 cm), and 10 *T. bactrae* adults were released inside. The mortality was observed at 24, 48, and 72 hours after treatment. Temperature was maintained at 25°C.

DBM and *Corcyra cephalonica* eggs were collected and fixed on the card (2 × 2 cm), and exposed to the UV light for 0.5 hour. Egg cards were put into the vial (2 × 10 cm) which contained *T. bactrae* adults. After parasitism, egg cards were dipped in insecticide solutions for 10 sec and the mortalities of eggs and emerged parasite adults were noted.

Results and Discussion

The numbers of eggs, larvae and pupae observed at different crop growth stages were recorded, and the total incidence of insects was calculated (Table 1).

In order to estimate the actual number (N) of insects in each stage, the following method (Kiritani and Hokyo, 1962) was used:

$$N = I.n/D$$

where:

N = Estimate the actual number
I = Census interval
n = Total incidence
D = Developmental period of each stage

The developmental period of the immature stage was estimated by the regression equation between the developmental velocity (V) and temperature (T). The calculated regression equations for each stage are as follows:

Egg; V (= 1/D) = 0.0120T 0.0155 (r = 0.993)
Larva; V (= 1/D) = 0.0065T 0.0574 (r = 0.966)
Pupa; V (= 1/D) = 0.0136T 0.1233 (r = 0.991).

Table 1. Estimation of the number of DBM at each stage using population monitoring data in naturally infested plots in three provinces, 1985-89.

Developmental stage	Petchaboon Province (January - December 1985) Period study									Nakornprathom Province (July 1987 - January 1988)								
	Mar. - Apr.			May - Jun.			Nov. - Dec.			July			Oct.			Jan.		
	D	n	N	D	n	N	D	n	N	D	n	N	D	n	N	D	n	N
Eggs	3.64	389	1068.68	3.64	964	2648.35	4.91	3824	7788.19	2.95	1582	1072.5	3.16	418	264.6	3.50	8432	4835.4
Larvae	10.02	564	562.87	10.02	542	540.92	16.33	5222	3197.80	7.44	825	221.8	8.15	277	68.0	9.45	4667	987.7
Pupae	4.86	77	58.44	4.86	40	82.31	8.01	1103	1377.02	3.60	146	81.1	3.95	67	33.9	4.59	1285	559.9
Temp.(°C)		24.19			24.19			18.26			29.5			27.7			25.1	

Developmental stage	Kanchanaburi Province (July 1988 - June 1989) Period study								
	Mar - Apr.			Jul. - Aug.			Nov. - Dec.		
	D	n	N	D	n	N	D	n	N
Eggs	2.77	521	1880.86	2.94	191	649.65	3.57	392	1098.03
Larvae	7.14	217	303.02	7.69	60	78.02	10.00	680	680.00
Pupae	3.33	39	117.12	3.70	4	10.81	4.76	133	279.41
Temp. (°C)		31.85			30.00			25.15	

D= Estimated developmental perod (D,Day); n = Incidence; N = Estimated number.

The life tables were developed on the basis of the estimated number of insects in each stage (Tables 2, 3 and 4). The number of living insects/plant in each stage (Ix) is given. The parasitism was evaluated from the rearing of collected insects. Since the actual emergence rate of adults was not observed in the field, the number of adults emerged may probably be overestimated. The survival rate of DBM from egg to adult emergence was highest in the dry season and lowest in the wet season. The low survival rate in the wet season may be due mainly to parasites and rainfall. Sivapragasam (1986) developed life tables of DBM at Nagoya, Japan. He evaluated the effect of rainfall on eggs and showed that 38% mortality resulted from wash-off of the eggs by the rain. The survival rates in the highland Khao Khor was high especially in the dry season and the hot season. The rates were 13.9% and 13.3% with average temperatures of 18.3°C and 24.2°C respectively. However, the survival rates in the lowland Kanchanaburi were high only in the dry season. The survival rates were low during the hot season (6.2%) with average temperature of 31.9°C. High temperatures in the hot season may affect the survival of immature stages and reduce the adult fecundity. The rates of egg hatching, pupation and adult emergence were relatively high at 17.5-27.5°C and low at 30 and 32°C (Koshihara 1986). In the highlands parasitism by *T. confusum* and *C. plutellae* was highest in the wet season, but that by pupal parasite (*D. collaris*) was highest in the dry season. In the lowlands parasitism by *T. bactrae* ranged from 16.2 to 45.2% and that by *C. plutellae* 6.1 to 32.4%. These rates were highest in the wet season.

From the life cycle of DBM at different crop growth stages, it was found that the life cycle in the cool dry season (Nov.-Dec.) was the longest (29.25 days) at Petchaboon Province, and the shortest in the hot season (Mar.-Apr.) (13.24 days) at Kanchanaburi Province. Estimated numbers of generations of DBM were equal to 17 and 25.5 per year at Petchaboon and Kanchanaburi provinces , respectively. The average lowest temperatures in the dry season (Nov.-Dec.) were 18.3°C and 25.2°C at Petchaboon and Kanchanaburi, respectively. The mean temperature year-round was 22.2 and 29.1°C at Petchaboon and Kanchanaburi, respectively. The wet season in Thailand lasts from May to October. The highest precipitation is during July August.

Table 2. Life table of DBM in a naturally infested plot at Petchaboon Province 1985.

x	dxF	Wet season May-June			Dry season Nov.-Dec.			Hot season Mar.-Apr.		
		lx	dx	%	lx	dx	%	lx	dx	%
Egg		110.35			324.51			44.53		
	Egg parasite[a]		17.05	15.45		7.21	2.22		0	0
	Unknown		70.76	64.12		183.98	66.69		21.08	47.34
	Total		87.81	79.57		191.19	68.91		21.08	47.34
Larva		22.54			133.24			23.45		
	Larval parasite[b]		4.82	21.38		15.14	11.36		3.49	14.89
	Unknown		14.29	63.40		60.72	45.57		13.36	56.97
	Total		19.11	84.78		75.86	56.93		16.85	71.86
Pupa		3.43			57.38			6.60		
	Pupal parasite[c]		0.17	4.96		12.03	20.98		0.77	11.69
	Unknown		?	?		?	?		?	?
	Total		≥0.17	≥4.96		≥12.03	≥20.97		≥0.77	≥11.69
Adult		≤3.26			≤45.35			≤5.90		
Survival rate (%) (Egg-Adult)				≤2.95			≤13.97		≤13.25	

[a] *Trichogramma confusum*; [b] *Cotesia plutellae*; [c] *Diadromus collaris*.

Table 3. Major mortality factors of DBM in naturally infested plot at Nakornprathom Province (1987-88).

x	dxF	Wet season May-June lx	dx	%	Dry season Nov.-Dec. lx	dx	%	Hot season Mar.-Apr. lx	dx	%
Egg		35.75			8.82			161.18		
	Egg parasite[a]		5.79	16.2		3.99	45.2		32.56	20.2
	Unknown		22.57	63.1		2.99	33.9		95.70	59.4
	Total		28.36	79.3		6.98	79.4		128.26	79.6
Larva		7.39			2.66			32.92		
	Larval parasite[b]		0.45	6.1		0.85	32.4		4.51	13.7
	Physiological death		0.89	12.1		0.67	25.5		2.50	7.5
	Unknown		3.35	45.3		0.01	0.4		7.25	22.0
	Total		4.69	63.5		1.53	58.3		14.26	43.2
Pupa		2.70			1.13			18.56		
	Physiological death		0.56	20.6		0.25	22.4		2.35	12.6
	Unknown		?	?		?	?		?	?
	Total		≥0.56	≥20.6		≥0.25	≥22.4		≥2.35	≥12.6
Adult		≤2.14			≤ 0.88			≤16.31		
Survival rate (%) (Egg-Adult)		≤5.9			≤10.0			≤10.2		

[a]*Trichogrammatoidea bactrae* Nagaraja; [b]*Cotesia plutellae* Kurdj.

Table 4. Major mortality factors of DBM in a naturally infested plot, Kanchanaburi Province (July 1988-June 1989).

x	dxF	Wet season I May-June lx	dx	%	Dry season II Nov.-Dec. lx	dx	%	Hot season Mar.-Apr. lx	dx	%
Egg		27.06			45.75			78.36		
	Egg parasite[a]		4.25	15.70		0.11	0.24		3.87	4.93
	Unknown		19.57	72.32		16.94	37.02		61.83	78.90
	Total		23.82	88.02		17.05	37.26		65.70	83.83
Larva		3.24			28.70			12.66		
	Larval parasite[b]		0.32	9.87		0	0		0.37	2.92
	Unknown		2.47	76.25		17.06	59.44		10.54	83.25
	Total		2.79	86.1		17.06	59.44		10.91	86.17
Pupa		0.45			11.64			1.75		
	Physiological death		0	0		0	0		0	0
	Unknown		?	?		?	?		?	?
	Total		≥0	≥0		≥0	≥0		≥0	≥0
Adult		≤0.45			≤11.64			≤1.75		
Survival rate (%) (Egg-Adult)				≤1.67			≤25.44			≤6.23

[a]*Trichogrammatids*, [b]*Cotesia plutellae*.

Table 5 shows that the percent mortality of adults of *T. bactrae* was high for phenthoate and benfuracarb but was low for fenvalerate, cycloprothrin, ethofenprox, *B. thuringiensis* and abamectin.

Additional experiments were performed in 1990 to include more insecticides used by farmers in some areas. Among those insecticides (chlorfluazuron, mevinphos, cartap and prothiofos), chlorfluazuron showed low toxicity to the adults of *T. bactrae*.

The toxicity of insecticides to parasitized egg and nonparasitized egg of *C. cephalonica* and DBM is shown in Table 6. Phenthoate and benfuracarb showed higher toxicity to parasitized eggs. The emergence of *T. bactrae* from parasitized DBM eggs was low in phenthoate, fenvalerate, ethofenprop and benfuracarb treatments. The results showed that cycloprothrin,

Table 5. Contact toxicity of the insecticides to the adult of *T. bactrae* (1989-90).

Insecticides	Dilution time	1989 % Mortality			Insecticides	Dilution time	1990 % Mortality		
		24	48	72 hours			24	48	72 hours
Phenthoate 50EC	200	42.5	97.5	100.0	Chlorfluazuron 5EC	200	5.0	5.0	7.5
	2000	7.5	17.5	45.0		2000	0	10.0	12.5
Fenvalerate 20EC	200	0	5.0	7.5	Cartap 50SP	200	55.0	80.0	100.0
	2000	0	5.0	10.0		2000	7.5	42.5	80.0
Cycolprothrin 10EC	200	0	2.5	10.0	Mevinphos 24EC	200	100.0	100.0	100.0
	2000	0	7.5	10.0		2000	62.5	92.5	100.0
Ethofenprox 20EC	200	17.5	22.5	27.5	Prothiofos 50EC	200	85.0	100.0	100.0
B. thuringiensis	2000	12.5	17.5	25.0		2000	15.0	65.0	85.0
A+K WP	200	2.5	5.0	5.0	Control	–	0	0	5.0
	2000	0	2.5	5.0					
Abamectin 1.8EC	200	0	0	7.5					
	2000	0	2.5	5.0					
Benfuracarb 20EC	200	45.0	75.0	97.5					
	2000	15.0	57.5	85.0					
Control	–	0	0	0					

Table 6. Toxicity of insecticides to unparasitized and *T. bactrae* parasitized eggs of *Corcyra cephalonica* and DBM.

Insecticides	Dilution time	1989 *C. cephalonica*		1990 DBM	
		% Egg parasite emerged from parasitized eggs	% Egg hatch from non-parasitized eggs	% Egg parasite emerged from parasitized eggs	% Egg hatched from non-parasitized eggs
Phenthoate 50EC	200	20.0	25.0	0	52.5
	2000	60.0	45.0	7.5	75.0
Fenvalerate 20EC	200	57.5	20.0	0	22.5
	2000	77.5	62.5	25.0	52.5
Cycolprothrin 10EC	200	77.5	57.5	62.5	47.5
	2000	72.5	70.0	77.5	75.0
Ethofenprox 20EC	200	42.5	10.0	5.0	50.0
	2000	72.5	25.0	5.0	82.5
B.t.A+K WP	200	65.0	55.0	77.5	72.5
	2000	72.5	77.5	95.0	90.0
Abamectin 1.8EC	200	57.5	27.5	77.5	65.0
	2000	70.0	52.2	85.0	87.5
Benfuracarb 20EC	200	25.0	0	0	15.0
	2000	30.0	0	0	70.0
Control	–	87.5	80.0	97.5	100.0

B. thuringiensis and abamectin had low toxicities, but phenthoate, fenvalerate, ethofenprop and benfuracarb had high toxicities to parasitized eggs of DBM.

It can be concluded that if these insecticides are used in the cabbage field, DBM larvae may or may not be killed depending upon whether or not DBM has developed resistance to these chemicals. However, some of these insecticides show low toxicity to egg parasites while others are highly toxic. Therefore, for effective control of DBM, the selective insecticides that are safer for the egg parasite should be carefully considered.

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Selection of Effective Species or Strains of *Trichogramma* Egg Parasitoids of Diamondback Moth

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Abstract

Twenty-seven *Trichogramma* and *Trichogrammatoidea* species or strains of different origin were tested on diamondback moth (*Plutella xylostella* (L.)) for host acceptance and host suitability. The experiments were conducted at 27 ± 2°C and 75-90% RH. Depending on their initial parasitization activity, the number of offspring produced and emergence ratio, 18 *Trichogramma* species were identified as parasitoids of diamondback moth. Twelve *Trichogramma* species were compared by host preference experiments. None of the *Trichogramma* species showed host preference for diamondback moth over its laboratory rearing host *Corcyra cephalonica* Stainton, but differences between the species were found. The parasitization rate of diamondback moth eggs ranged between 0.47 for *Trichogramma semblidis* and 25.67 for *Trichogrammatoidea bactrae*. Seven out of twelve *Trichogramma* and *Trichogrammatoidea* species (*T. bactrae*, *Trichogramma* sp. (France), *T. principium*, *T. pretiosum*, *T. leptoparameron*, *T. chilonis*, *T. confusum*, *T. ostriniae*) showed promise for further investigation into selecting a biological control agent against diamondback moth. Three *Trichogramma* species were checked in cage experiments. *T. pretiosum* showed good searching capacity and percentage of parasitism (9.45/34.09%) compared to *Trichogramma* sp. (France) (2.5/19.26%) and *T. leptoparameron* (0.125/2.5%).

Introduction

Trichogrammatids are egg parasitoids of a large number of insect species. *Trichogramma chilonis* Ishii was found on the eggs of diamondback moth (DBM) (*Plutella xylostella* (L.)) (Lepidoptera: Yponomeutidae) by Okada (1989) in Japan, and *Trichogrammatoidea bactrae* Nagaraja on the eggs of DBM in farmers' fields in Thailand. Both species were checked in the laboratory on DBM at AVRDC in Taiwan. Parasitization rate was satisfactory. Under high parasitoid density every DBM egg was parasitized. On plants and in the field, however, parasitism was not sufficient (Klemm, unpublished data). A selection of more efficient trichogrammatids, it was thought, might be promising in controlling DBM.

Pak (1988) tested 60 *Trichogramma* species/strains on five lepidopterous pests of cabbage. Hassan (1988) developed a laboratory glass tube method to select suitable *Trichogramma* species/strains to control the codling moth *Cydia pomonella* L., and two summer fruit tortrix moths *Adoxophyes orana* and *Pandemis heparana* (Lepidoptera: Tortricidae). Hassan and Guo (1991) used this method for selection of effective *Trichogramma* species or strains of egg parasitoids to control the European corn borer (*Ostrinia nubilalis* Hübner), and developed a simple cage test method to examine searching capacity.

The identification and selection of *Trichogramma* species/strains to control DBM had not yet been conducted. For this reason 27 *Trichogramma* species/strains were compared for their effectiveness in controlling DBM at Guangdong Entomological Institute (GEI), by utilizing laboratory glass tube and cage experiments. The identification of trichogrammatids as parasitoids of DBM was conducted by using a direct observation method, evaluating the initial parasitization activity, the ability for developing in DBM eggs and the emergence ratio of adult parasitoids. The host preference was compared by offering to a single trichogrammatid female the choice between eggs of two hosts, DBM and *Corcyra cephalonica* Stainton, respectively in a glass tube test. The parasitization rate was tested under 'no choice' conditions on DBM eggs. A cage experiment was conducted to evaluate the searching capacity of three *Trichogramma* species on DBM eggs on cabbage plants.

Materials and Methods

Insect cultures

The following 25 *Trichogramma* species/strains of exotic and indigenous origin were available at GEI: *T. agrotidis* (France), *T. cacoeciae* (USSR), *T. chilotraea* (Thailand), *T. confusum* (China), *T. cordubensis* (Iran), *T. deion* (USA), *T. dendrolimi* (China), *T. embryophagum* (France), *T. embryophagum* (Germany), *T. evanescens* (Iran), *T. japonicum* (China), *T. leptoparameron* (USSR), *T. maidis* (France), *T. nagarkatti* (France), *T. nubilale* (USA), *T. ostriniae* (China), *T. papilionis* (Japan), *T. pintoi* (USSR), *T. pretiosum* (USA), *T. principium* (USSR), *T.* sp. France (France), *T.* sp. USSR (USSR), *T. telengai* (USSR), and *T. trjapitzini* (USSR).

Two strains were provided by AVRDC: *Trichogramma chilonis* (Taiwan) and *Trichogrammatoidea bactrae* (Thailand).

A DBM culture was established from field-collected specimens. They were reared on Chinese cabbage (*Brassica campestris* ssp. *chinensis* L.) in 50 × 50 × 50 cm fine mesh nylon net cages. For DBM egg production, adults were confined in a container lined with filter papers dipped in cabbage leaf extract.

A culture of *C. cephalonica* was established at GEI. The sterilized eggs of this insect were used for rearing all *Trichogramma* and *Trichogrammatoidea* species/strains. Fresh eggs were continuously available. *Trichogramma* and *Trichogrammatoidea* species/strains were reared in 10 × 4 cm diameter glass tubes. All observations were conducted at 27±2°C, 75-90% RH.

Direct observation method

Host acceptance was observed in the first monitoring after description of typical oviposition behavior described by Pak (1988) and determined by the number of oviposition acts within one hour of insect release. The host egg:parasitoid ratio was 1:10-15. DBM eggs (100-200/card) were replaced four times during 1 hour to avoid superparasitism. Up to five oviposition acts per replication were counted to judge host acceptance, i.e. the activity of parasitization on DBM eggs. After 1 hour, DBM eggs were replaced by eggs of *C. cephalonica* for 15 min, to ensure that *Trichogramma* laid eggs in them and thus exclude the influence of factors other than the host species themselves on parasitization. Five days later the second monitoring was conducted by counting parasitized DBM eggs. Parasitized eggs turn black indicating the development of parasitoids. The parasitoid adult emergence ratios were evaluated 10 days after oviposition in a third monitoring. The latter two parameters were used to evaluate the host suitability of DBM for *Trichogramma* species/strains tested, i.e. the ability of development and emergence of trichogrammatids in/from DBM eggs.

Laboratory glass tube test methods

The host preference of *Trichogramma* was tested by offering the parasitoid the choice between eggs of DBM and *C. cephalonica* in glass tubes (Hassan 1988; Hassan and Guo 1991). A single *Trichogramma* female (12-24 hours after emergence) was released in a glass tube containing 80 eggs (2 × 40) of DBM and 80 eggs (2 × 40) of *C. cephalonica*.

Host eggs were glued on the surface of a small piece of paper (2 × 2 cm) and a drop of honey was placed at the center. To isolate a single female, *Trichogramma* adults were scattered on a smooth surface and an individual female was captured by placing an open end of a tube (50 × 9 mm diameter) around one individual. *Trichogramma* walked up over the tube walls and was easy to examine using a binocular. The single female is then transferred to the larger test tubes containing host eggs, by placing a suitable light source at the closed end of the large tube. The light stimulates the insect to walk towards the big tube. The adult female observed in the first monitoring was left with the host eggs until the second monitoring was conducted.

Host preference was monitored by (1) checking all tubes eight times during the first 6 hours of the experiment and recording the location of the parasitoid (on DBM or *C. cephalonica* or elsewhere); a minimum of 45 min elapsed between any two observations; (2) counting the number of parasitized eggs 5 days after release of females into the glass tubes, when the parasitized host eggs turned black.

Observations on the location of the parasitoid showed a preference by *Trichogramma* to search for, contact and remain on the eggs of its hosts. The number of parasitized host eggs/female (parasitization rate) shows the preference of the parasitoid female for laying eggs. The test should be repeated at least 30 times for each combination of *Trichogramma* and *Trichogrammatoidea* species/strain and host eggs.

The parasitization rate on DBM eggs (no-choice experiment) was tested in glass tubes. Separated *Trichogramma* females were provided by 80-100 DBM eggs on egg cards. Females were not fed. Five days later the parasitized eggs were counted.

Cage experiments

The searching capacity of *Trichogramma* species/strains was tested by releasing 20 adult female parasitoids in cages (50 cm × 50 cm × 50 cm). Each cage contained five potted cabbage plants. The plants were exposed for one night for DBM egg-laying. DBM eggs were naturally distributed on the upper and lower surface of the plants. About 100 of these eggs per cage were marked. In addition, DBM eggs laid on paper were glued on the upper surface of the cabbage plant. Parasitized DBM eggs which had been previously marked were counted and percent parasitism was calculated. The number of parasitized eggs, divided by the number of released *Trichogramma* females, represents the searching capacity. The method was adapted from that of Hassan and Guo (1991).

Results and Discussion

Direct observation method

Nine of the tested species/strains did not show any oviposition behavior on DBM eggs, although these eggs were contacted at high frequency by *T. cacoeciae*, *T. chilotraea*, *T. cordubensis*, *T. embryophagum* (France), *T. embryophagum* (Germany), *T. evanescens* (Iran), *T. evanescens* (USSR), *T. maidis* and *T. papilionis*. Each of these species/strains readily parasitized eggs of *C. cephalonica*. Based on the criteria of host acceptance and suitability, 18 out of 27 tested *Trichogramma* species/strains were identified as parasitoids of DBM (Table 1). The 18 species were ranked after the first sequence of experiments, depending on their initial parasitization activity, number of eggs parasitized and emergence ratio of the offspring.

Distinct differences on these parameters were found among the 18 species. Eleven species showed high (more than 20 oviposition acts/hour), two species medium (11-20 oviposition acts/hour) and five species low (1-10 oviposition acts/hour) initial parasitization activity.

Whenever significant oviposition behavior was observed, the parasitoids were able to develop in, and to emerge from, DBM eggs. The emergence ratio ranged between 27.66% (*T. telengai*) and 100% (*T. agrotidis* and *T. nubilale*). A varying number of developing offspring was counted. Four species showed a high (more than 100 eggs parasitized), five species a medium (51-100 eggs parasitized) and nine species showed a low (1-50 eggs parasitized) level of parasitization of DBM eggs during 1 hour of observation. When parasitic activities and number of progeny produced were similar, the parasitoid species were ranked by the level of progeny emergence ratio.

Table 1. Ranking of DBM egg parasitoids according to parasitization activity, production of offspring and emergence ratio of adult parasitoids.

Rank	Strain	Origin	Parasitism activity[a]	Production of offspring[a]	Emergence (%)
1	*T. bactrae*[b]	Thailand	3	3	97.04
2	*T. principium*	USSR	3	3	96.24
3	*T. sp.* (France)	France	3	3	93.67
4	*T. confusum*	China	3	3	86.10
5	*T. sp.* (USSR)	USSR	3	2	94.10
6	*T. leptoparameron*	USSR	3	2	91.67
7	*T. deion*	USA	3	2	90.00
8	*T. pintoi*	USSR	3	2	76.04
9	*T. chilonis*	Taiwan	3	2	72.94
10	*T. nagarkatti*	France	3	1	61.90
11	*T. telengai*	USSR	3	1	27.66
12	*T. pretiosum*	USA	2	1	82.50
13	*T. semblidis*	USSR	2	1	81.25
14	*T. nubilale*	USA	1	1	100.00
15	*T. agrotidis*	France	1	1	100.00
16	*T. trjapitzini*	USSR	1	1	90.62
17	*T. ostriniae*	China	1	1	90.33
18	*T. japonicum*	China	1	1	47.05

[a]1 = low, 2 = medium, 3 = high. [b]belongs to the genus *Trichogrammatoidea*; all other species to the genus *Trichogramma*.

Laboratory glass tube tests

The species ranked 1-9, 12, 13 and 17 were checked for their host preference. An ideal *Trichogramma* species should be active in searching for and showing parasitization preference for DBM eggs. None of the species was so ideal, but certain differences were observed.

The number of contacts per female on the eggs of DBM and *C. cephalonica* after 8 observations, as well as the resulting number of parasitized eggs after 5 days are shown in Fig. 1. None of the species showed host preference in searching for or parasitization of DBM eggs. This might be caused by the small size of DBM eggs (Pak 1991). Distinct preferences in searching for *C. cephalonica* were shown by *T. confusum* (4), *T. deion* (7), *T. pintoi*, (8), *T. chilonis* (9), *T. pretiosum* (12), *T. semblidis* (13) and *T. ostriniae* (17). *Trichogramma* sp. (France) (3) showed best results in searching for, and parasitization of, DBM eggs (1.6 contacts/female; 12.23 DBM eggs/female). The parasitization rate was 38.9 eggs/female. *Trichogrammatoidea bactrae* (1) and *T. pretiosum* (12) were less active in searching for DBM eggs (0.8 and 0.5 contacts of DBM eggs/female), and parasitized an average of 7.93 and 7.13 DBM eggs/female. *Trichogramma leptoparameron* (6), *T. principium* (2) and *T. pintoi* (8) had 1.53, 1.13 and 1.07 contacts/female on DBM eggs, but were less active in parasitization (4.3, 6.6 and 0.43 parasitized

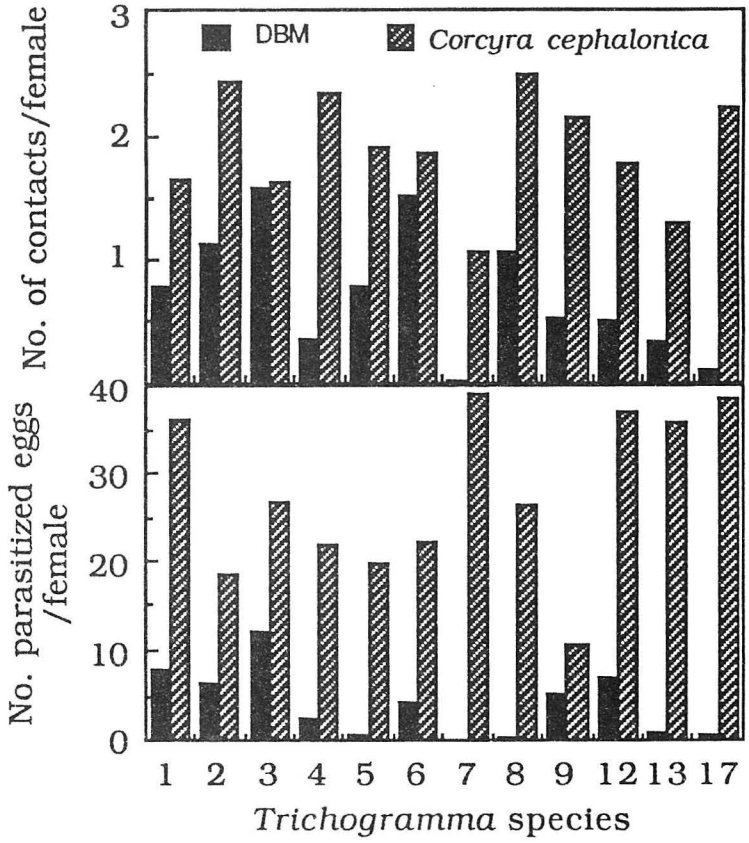

Fig. 1. Results of the laboratory glass tube test on host preference in searching for (No. of contacts/female) and parasitization (No. of parasitized eggs/female) of the eggs of DBM or *C. cephalonica* by various *Trichogramma* species. See Table 1 for identification of species.

DBM eggs/female). The number of parasitized host eggs was 26.5, 25.3 and 26.8 host eggs/female, respectively. The highest number of parasitized host eggs was presented by *T. bactrae* (1) (44.2) and *T. pretiosum* (12) with 44.03 eggs/female.

The correlation between the number of contacts/female of the tested species on DBM eggs and the resulting number of parasitized DBM eggs is shown in Fig. 2. Searching for DBM eggs leads to its parasitization by *Trichogramma* sp. (France) (3) and *T. principium* (2); *T. leptoparameron* (6) was searching for DBM eggs but parasitization was low. *Trichogrammatoidea bactrae* (1). *T. pretiosum* (12) and *T. chilonis* (9) showed less searching activity for DBM eggs than *T. pintoi* (8), but higher numbers of parasitized DBM eggs/female. The number 0.8 contacts on DBM eggs/female by *Trichogramma* sp. (USSR) (5) and *T. bactrae* (1) results in a difference of 7.3 parasitized DBM eggs in favor of *T. bactrae* (1).

The parasitization rate on DBM eggs (no choice experiment) is shown in Fig. 3. *Trichogrammatoidea bactrae* parasitized 25.67 DBM eggs/female, followed by *T. principium* (2) (24.27 eggs/female). *T. pretiosum* (12) (18 eggs/female), *T. ostriniae* (17) (12.5 eggs/female), *T.* sp. (France) (11.67 eggs/female), *T. chilonis* (9) (11.43 eggs/female) and *T. confusum* (4) (11.23 eggs/female), respectively. All other species parasitized less than 10 eggs/female.

Based on their performance on host preference and parasitization of DBM eggs (no choice experiment), 7 out of 12 tested species were identified as suitable for further investigations: *T. bactrae* (1) was confirmed to be one of the best species, as well as *Trichogramma* sp. (France) (3), *T. principium* (2), *T. pretiosum* (12), *T. chilonis* (9), *T. ostriniae* (17) and *T. leptoparameron* (6), respectively. To select the most efficient species for the control of DBM in farmers' fields, performance on searching capacity on cabbage plants was evaluated next.

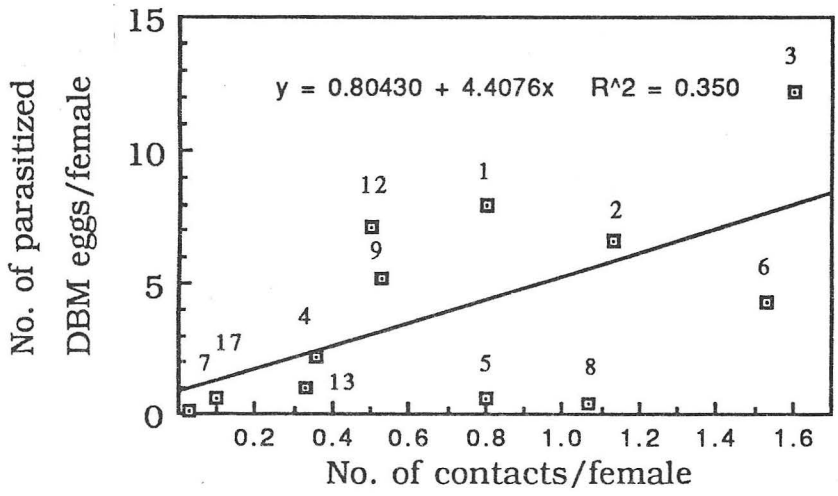

Fig. 2. Correlation between number of contacts of *Trichogramma* females with DBM eggs and number of eggs parasitized. Laboratory glass tube test on host preference. The numbers indicate the serial number of *Trichogramma* species listed in Table 1.

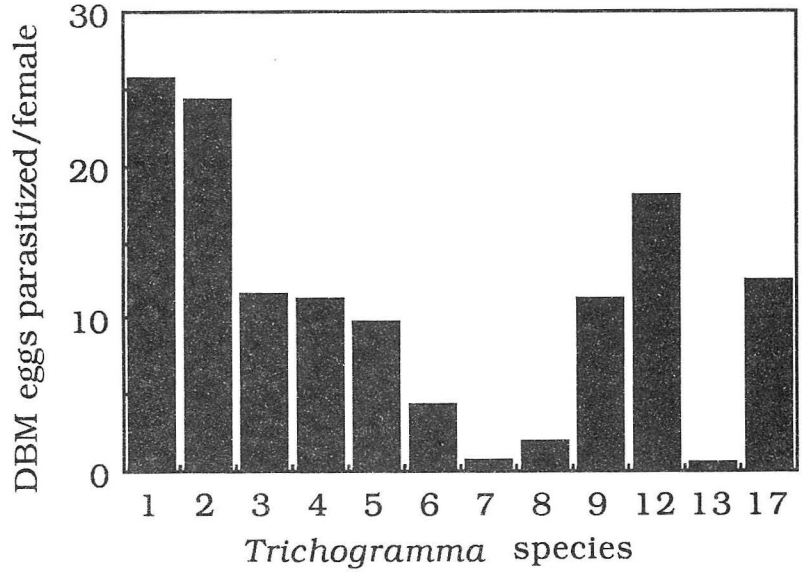

Fig. 3. Results of a laboratory glass the test on parasitization of DBM eggs by *Trichogramma* species. For species identification see Table 1.

Plant cage experiments

Three out of 18 identified *Trichogramma*/*Trichogrammatoidea* species were compared using cage experiments for their ability to search for and parasitize DBM eggs on cabbage plants. *Trichogramma pretiosum* showed the highest percentage of parasitism (34.09%) of DBM eggs on cabbage plants and the highest searching capacity (9.45 eggs parasitized by one female) (Table 2). *Trichogramma* sp. (France) had 19.26% parasitism and 2.5 parasitized eggs per female. *Trichogramma leptoparameron* showed very low parasitism (2.48%) and an average number of only 0.125 parasitized DBM eggs per female. Marking DBM eggs on cabbage plants was suitable to judge the efficiency of parasitism by trichogrammatids.

Table 2. Searching capacity and parasitism by *Trichogramma* of DBM eggs on cabbage plants. Cage experiment.

Species	No. of parasitized DBM eggs/female (±SD)	Parasitism %
T. sp. (France)	2.5 ±1.24b	19.26
T. pretiosum	9.45±3.47a	34.09
T. leptoparameron	0.13±0.25b	2.48

Means followed by the same letter are not significantly different at the level of 5%, Duncan's Multiple Range Test (Duncan 1955).

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CHEMICAL CONTROL

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Control of Diamondback Moth by Application of Neem Extracts

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Abstract

Some extracts from neem seeds/neem seed kernels can give satisfactory to good control of diamondback moth, *Plutella xylostella* (L.), in cabbage and other crucifers. Water extracts prove most effective, sometimes even at low concentrations as low as 12.5 g of seed kernels per liter of water, provided the raw material contains sufficient azadirachtin. Neem could be developed into products suitable for IPM in cabbage, owing to their selectivity towards the natural enemies of pests. Neem application in cabbage can also have negative effects, such as a change of plant color and reduction of head-size. These disadvantages are considered of minor importance if heavy damage or even complete loss of the crop could be prevented by neem application. The use of neem seed kernel water extract is primarily of interest to the small vegetable farmers in developing countries as they can produce their own pesticide using locally available tools and raw material. The delayed effect of neem products, especially at low temperatures, may discourage farmers who are used to the quick impact of synthetic contact insecticides. The farmers' resistance to slower acting products could be overcome by training. There seems to be no danger of diamondback moth developing resistance to neem products in the short term.

Introduction

The identification of selective insecticides is urgently needed for successful integrated pest management, especially in crucifers, because the synthetic insecticides can be harmful to the natural enemies, and because pest insects like diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera:Yponomeutidae), develop resistance to them.

Although DBM is the dominant pest in many parts of the world, it is usually not the only insect harmful to cabbage and related vegetables. It is usually one of a complex of pests consisting of various species from different orders and families. Some members of these pest complexes may be controlled successfully by using natural enemies. To control all important pests of crucifers, the use of insecticides may become necessary. Selective products should be the ones used, however.

Some components of the widely distributed tropical neem tree, *Azadirachta indica* A. de Jussieu, especially those from seeds/seed kernels, proved effective against numerous insect pests, in particular against the larvae of Lepidoptera. Nevertheless, the components are selective, especially towards parasitoids, and under field conditions also towards predators.

Over the last 12 years, the ability of neem products to control DBM and other crucifer pests has been studied in field trials of various individuals working on our research project (Natural Pesticides from Tropical Plants). Some of the results obtained in Africa, Asia and Latin America are summarized below.

Materials and Methods

Leaf extracts and seed and kernel extracts of neem were used in the trials. The latter were preferred since the ingredients of the seeds/seed kernels, especially azadirachtin, were clearly more effective than those of the leaves.

Feuerhake and Schmutterer (1982), Feuerhake (1984), Adhikary (1985), Dreyer (1986, 1987) and Kirsch (1986, 1987) present detailed descriptions of the production of the various extracts. These researchers used not only aqueous extracts but also methanolic extracts, but in the interests of the small-scale vegetable farmers in developing countries being able to produce their own neem pesticides, the former were clearly preferred.

The seeds of neem used in Africa originated in various regions of Togo, and contained about 3.1-6.2 μg azadirachtin/g of seed kernel. The seed kernels extracted in the Philippines were imported from Indonesia (central parts of Java; azadirachtin content: 4.75 μg). In Latin America (Dominican Republic) the neem material originated from Haiti and a small plantation in San Cristóbal; its azadirachtin content was about 3.56-3.84 μg/g. All experiments were fully randomized blocks with at least four replicates per treatment.

The application of the neem products was made in one trial in Togo by means of a watering can, otherwise by using an ordinary knapsack sprayer. In all trials several treatments were applied at weekly intervals. As a rule, 4-5 treatments were applied per cropping season.

Results

Experiments in Africa

The results of two experiments in southern Togo (Adhikary 1985) are given in Tables 1 and 2. The first experiment (Table 1) was run in 1979, the other one (Table 2) in 1981. In the first trial neem leaf powder extracted with water was used, as were methanolic neem leaf extracts and methanolic seed kernel extracts. For comparison mevinphos, an organophosphorus insecticide, was also included in the experiment. In the second trial only methanolic leaf and seed kernel extracts in two concentrations (2 and 4%) were applied, along with the synthetic pyrethroid deltamethrin.

Table 1. Effect of treatments with neem extracts and mevinphos on cabbage yields and infestation by DBM in 1979 (Adhikary 1985).

Treatments	Yield (t/ha)	% Damaged heads	No. DBM larvae + pupae/ head
Control (Estravan + 2% methanol)	30.5	77	4.6
NLP-water suspension by watering can 4%	31.1	78	5.0
NLP-M 4%	36.4	65	2.9
NKP-M 4%	38.3	9	0.9
NKP-M 2%	38.8	27	1.5
Mevinphos 0.05%	38.2	19	1.7
SE	1.8	6.8	
SE between means of treatment	2.5		
F-value	4.3**	25.4***	

NLP = Neem leaf powder; NLP-M = Methanolic neem leaf powder extract; NKP-M = Methanolic neem seed kernel powder extract. Data are means of four replicates.

Table 2. Effect of treatments with crude methanolic neem leaf and seed kernel extracts and deltamethrin on cabbage yields and infestation by DBM in 1981 (Adhikary 1985).

Treatments	Yield (t/ha)	% Damaged heads	No. DBM larvae + pupae/ head
Control I (Citowett + 2% methanol)	44.2	36.7	3.5
Control II (Citowett + 2% methanol) plots outside trial	26.7	52.6	25.0
NKP-M 4%	50.8	10.1	1.9
NLP-M 4%	37.5	16.1	2.9
NKP-M 2%	51.1	4.3	1.1
NLP-M 2%	46.3	41.6	3.0
Deltamethrin 0.2%	47.2	0.0	0.7
SE	0.60	4.36	2.09
CD	1.85	13.44	6.44
F	5.52**	21.9***	17.2**

NLP-M = Methanolic neem leaf powder extract; NKP-M = Methanolic neem seed kernel powder extract. Data are means of four replicates.

In the first trial, the aqueous leaf powder extract applied by watering can and the methanolic leaf extract (4%) showed the lowest efficacy in reducing the percentage of cabbage heads damaged by DBM whereas the two methanolic neem seed kernel extracts and mevinphos showed significant improvement over the control. The highest concentration (4%) of the methanolic seed kernel extract was the most effective. As far as yield was concerned, the two variants of neem seed kernel extracts, the leaf extracts and mevinphos were significantly more effective than the control.

In the second trial deltamethrin (Decis) treatment resulted in no damage by DBM whereas in the two variants with methanolic neem seed kernel extracts (2 and 4%) 4.3 and 10.1% damaged cabbage heads were counted respectively. The two variants treated with methanolic neem leaf extracts gave no significant increase in yield. On the other hand the treatments with two concentrations of the methanolic seed kernel extract increased the yield significantly. Additional control plots outside the trial (Table 2) showed by far the highest percentage of damaged heads and the lowest yield.

The results of these experiments show that application of methanolic neem seed kernel extracts controls DBM satisfactorily under Togo conditions. The differences in efficacy between the neem kernel extracts and the synthetic pesticides, such as mevinphos and deltamethrin, were not striking.

Further trials on white cabbage and cauliflower in Togo were conducted by Dreyer (1987). The pyralid moth *Hellula undalis* (F.) appeared as an additional major cabbage pest in these experiments. By boring into the growing point this pest may cause the complete loss of young plants.

In the first experiment with white cabbage the following products were applied: three concentrations of aqueous neem seed extract (12.5, 25 and 50 g seed/l of water), the *Bacillus thuringiensis* product Dipel (300 and 900 g product/ha), the formulated methanolic neem seed kernel extract AZT-VR-K-EC (300 ppm/l; Feuerhake 1984) and a combination of Dipel (300 g product/ha) and aqueous neem seed extract in low concentration (12.5 g/l). The most important findings of this trial are given in Table 3.

In the control plots numerous plants were lost, mainly through attack by *H. undalis*. There were also some losses in the treatment plots, particularly in those with Dipel and AZT-VR-K-EC. The combination of Dipel with neem seed extracts in low concentrations gave a much better result than Dipel alone. The three treatments with neem water extracts gave the best results.

The level of damage to cabbage heads at harvest time (Table 3) was least with the aqueous neem seed extracts, with some variation depending on the concentration applied. The order of

efficacy of the other treatments was: the Dipel + water extract combination, the two variants of Dipel alone (300 and 900 g/ha), and, finally, the treatment with AZT-VR-K-EC, which did not differ much from the Dipel treatments. Owing to additional damage by *H. undalis* only a few plants/heads remained in control plots. These heads all had moderate to heavy damage (Table 3). Most heads in the other treatments were damage-free or had moderate damage on outer leaves, and all these heads were marketable.

In the trial with cauliflower (Table 4) only three variants with different concentrations (12.5, 25 and 50 g of seed kernels/l of water) were compared with each other and with a control. As in the other experiment, heavy crop losses were caused by DBM and *H. undalis* in the untreated plots. By far the most quality heads were harvested from the three neem treatments (Table 4) There was no significant difference between them.

Table 3. Results of a trial against *Plutella xylostella* in white cabbage in Togo.

Treatments	No. of plants/plot level of damage[a]			Sum	Mean of damage	Destroyed plants
	1	2	3			
Control	0	3.3	2	5.5	2.6 a[b]	9.7
WE 12.5 g NS/l	16.0	7.7	0	23.7	1.3 de	6.3
WE 25 g NS/l	24.3	1.3	0	25.6	1.1 e	5.7
WE 50 g NS/l	24.7	1.3	0	26.0	1.0 e	7.0
AXT-VR-K-EC 300 ppm/l	2.0	15.7	0	17.7	1.9 bc	9.7
Dipel 900 g/ha	1.0	12.7	0.3	14.0	1.9 bc	4.0
Dipel 300 g/ha	1.0	12.3	0	13.3	1.9 bc	8.0
Dipel 300 g/ha + WE 12.5 g NS/l	11.7	12.3	0	24.0	1.5 cd	7.0

[a]Levels of damage: 1 = plants without damage; 2 = plants with medium damage, specially on old leaves, restricted marketability; 3 = plants with heavily damaged old and young leaves, unmarketable.
[b]Within columns, means followed by a common letter are not significantly different at P = 1%.
WE = Water extract, NS = Neem seeds, AZT-VR-K-EC = Formulated, methanolic, neem seed kernel extract.
(Source: Dreyer 1986.)

Table 4. Results of a trial against DBM and *Hellula undalis* in cauliflower in Togo.

Treatments	No. of heads/plot with level of damage[a]			Sum	Mean damage	Mean of weight/head (g)
	1	2	3			
Control	4.0	4.0	1.5	9.5	1.7 a[b]	225 a[b]
WE 12.5 g NS/l	20.5	0.3	0	20.8	1.1 b	290 a
WE 25 g NS/l	22.3	0	0	22.3	1.0 b	285 a
WE 50 g NS/l	23.0	0	0	23.0	1.0 b	295 a

[a]Levels of damage: 1 = head white and clean; 2 = head brownish (lack of shade), slightly or not contaminated by frass, restricted marketability; 3 = head dark (lack of shade) and heavily contaminated by frass, unmarketable.
[b]Within columns, means followed by a common letter are not significantly different at P = 1%.
WE = Water extract from neem seeds (NS). (Source: Dreyer 1986).

Experiments in Asia

In the Philippines, specifically in the highlands of northern Luzon at Baguio, a trial was conducted on cabbage to control DBM, the main pest in the region (Kirsch 1987). In addition to DBM, *Pieris canidia* (Sparrm.) (Lepidoptera:Pieridae) and flea beetles of the genus *Phyllotreta* (Coleoptera:Chrysomelidae) occur as occasional pests. Control measures against DBM by local

farmers are characterized by frequent applications of cocktails of insecticides, often at very short intervals of about 3-4 days. This has led to widespread resistance not only to all major groups of pesticides but also to acylureas and *Bacillus thuringiensis* products (Kirsch and Schmutterer 1988).

Aqueous neem seed kernel extracts (25 g seed kernel/l of water), the *B. thuringiensis* product Thuricide HP (1 kg/ha), a combination of the water extract (25 g/l) and Thuricide HP (1 kg/ha), the formulated methanolic neem seed kernel extract AZT-VR-K-EC (0.2%), the synthetic pesticide profenofos and the acylurea Bayer SIR 14591 250 EC (50 g ai/ha) were used (Table 5).

The number of small and large larvae of DBM 6 days after treatment was not greatly reduced except by Bayer SIR 14591, but an almost complete collapse of the treated populations was observed before pupation, leading to very few remaining prepupae and pupae in the neem treatments (Table 5). Thuricide and profenofos were not sufficiently effective.

The plots treated with Bayer SIR 14591 showed much greater yields than all other treatments, followed by AZT-VR-K-EC alone and the combination of the latter with *B. thuringiensis*. The seed kernel water extract alone and its combination with Thuricide HP were next in efficacy. Thuricide HP and profenofos showed the lowest efficacy owing possibly to resistance of DBM to these pesticides.

Results were similar for the damage of DBM on cabbage heads (Kirsch and Schmutterer 1988). Bayer SIR 14591 was the most effective product, followed by the aqueous neem seed kernel extract and its combination with Thuricide HP, then AZT-VR-K-EC and its combination with Thuricide HP. Profenofos and Thuricide HP alone were not statistically different from each other nor from the control.

Table 5. Effect of neem extract and conventional insecticide treatments on the incidence of DBM (pupae) in cabbage (Kirsch 1986).

Insecticide, concentration or applied amount	Number of pupae[a]/10 plants 6 days after application no.		
	3	4	5
Control	4.8 a[b]	81.8 a[b]	44.0 a[b]
Profenofos 500 EC, 0.05% AI	1.5 ab	38.5 a	53.5 a
BAYER SIR 14591 250 EC, 50 g AI/ha	0.3 ab	0.5 cd	0 d
Thuricide HP, 1 kg/ha	0 b	17.8 b	72.2 a
AZT-VR-K EC, 0.2%	0 b	2.0 c	3.6 b
AZT-VR-K EC, 0.2% + Thuricide HP, 1 kg/ha	0.3 ab	0.3 cd	1.2 bc
ANSKE, 25 g NSK/l	0.3 ab	0 d	0.7 c
ANSKE, 25 g NSK/l + Thuricide HP, 1 kg/ha	0 b	0.3 cd	0.6 cd

[a]No pupae found after the first and second application. [b]Statistical analysis was based on values transformed by the log transformation. Backtransformed means are presented. Within columns, means followed by a common letter are not signifiantly different at P = 0.05 (Duncan's Multiple Range Test). ANSKE = Aqueous neem seed kernel extract, AZT-VR-K-EC = Formulated, methanolic neem seed kernel extract.

Experiments in Latin America

A number of trials were run in 1987-88 in Latin America (island of Hispaniola, Dominican Republic) to control DBM (Dreyer, unpubl. results) which is the dominant cabbage pest not only in the low-lying areas of the Dominican Republic but also in the central mountain range. The pyralid moth *Hellula philidealis* (Walker) is another major pest, as is the false cabbage aphid *Lipaphis erysimi* (Kaltenbach).

Despite the lack of detailed evaluation of these experiments, they provided some interesting findings. In three trials water extracts from seed kernels (25 and 50 g seed kernel/l of water) were applied. Dipel (product based on *B. thuringiensis*) at the recommended rate was also included in the trials for comparison.

The aqueous neem seed kernel extracts proved effective against DBM and *H. philidealis*, especially the higher concentrations. Dipel also gave good results against DBM. However, the aphid *L. erysimi* that appeared later during the season was not affected, and it appeared in such large numbers that all plants in the Dipel-treated plots dried up. Neem-treated plots (3.6 x 14.0 m) yielded on the average 52 kg of cabbage heads.

In the experimental field of the Instituto Politécnico Loyola in San Cristóbal (Dominican Republic) white cabbage has been grown during the winter since 1988, and protected by several sprayings of water extracts of neem seeds (50 g seed/l of water). A few holes caused by DBM were found, usually in the older leaves, but the heads were, with few exceptions, suitable for human consumption.

Discussion

The results of trials in Africa, Asia and Latin America have clearly shown that DBM and other important cabbage pests like *Hellula* spp. can be controlled through the application of neem extracts. This includes populations with high degrees of resistance to synthetic pesticides. Complete prevention of damage could not be achieved; such results would require extraordinarily careful sprayings of the infested plants, including their lower leaves.

In all three continents water extracts from seed kernels (seed kernel with hard shell) or seeds gave the best results. In Togo this was true even with such low concentrations as 12.5 g seed kernels/l of water. They were superior to a methanolic, formulated seed kernel extract (AZT-VR-K-EC), in spite of the relatively high concentration applied (0.2%). This at least partly depended on the amount of azadirachtin (the main active ingredient) in the extracts. Water can extract azadirachtin within 4-6 hours in considerable quantities. Water extracts can be produced by small-scale vegetable farmers with locally available tools, provided sufficient neem seeds of good quality and water are available.

It is of interest that in the trial in the Philippines the number of DBM larvae did not greatly decrease up to the sixth day after the application of neem (Table 5). This is typical for neem products, which show delayed effects, and also for regions with lower temperatures or at higher altitudes. Nonetheless it should be borne in mind that the food consumption by treated larvae is greatly reduced. Most of them die before pupation after an extended period of development (Table 5). In addition, surviving adults have a much lower fecundity than controls, usually less than 50%. This may lead to a gradual reduction of DBM populations after treatment of some generations. This reduction was observed in the Mandalay area in Myanmar (Burma) in 1988 (K. J. Feuerhake, personal communication).

On the other hand, the delayed effect of neem products makes them suitable for integrated pest management programs against DBM and other cabbage pests because this delay may give the parasitoids a chance to develop within their hosts without being harmed. Egg-parasitoids are also not killed by neem inside the eggs of Lepidoptera (Joshi et al. 1982). Adults of parasitoids (Hymenoptera) and spiders normally tolerate neem products in very high concentrations which exceed by far those used under field conditions. There is also, as a rule, no obvious influence

on the fecundity of neem-treated adult parasitoids, which means that the botanical is very selective to them.

In the short term, e.g. in a few years, there seems to be no danger of the development of resistance to neem products. Over 40-60 generations of two pesticide-resistant strains of DBM, kept under selection pressure of neem products in the laboratory, only a slight, insignificant reduction in susceptibility to neem was observed. This could not be explained with certainty as a first sign of resistance (Völlinger 1987). Nonetheless a purely neem-based sequence of sprayings for controlling DBM is not recommended, because it is not unlikely that this highly adaptive insect may also adapt to even a natural product like neem after some time under such conditions (Schmutterer 1990).

The development of an IPM program seems to be the best way to prevent or at least to postpone resistance problems. In addition to neem it could include products based on *B. thuringiensis* (if still effective) and parasitoids, perhaps also pathogens, and, if unavoidable, some synthetic pesticides with selective properties.

It should be pointed out that the use of neem for cabbage pest control does have disadvantages. In particular the oil-containing products, including water extracts, may damage the waxy layer of the leaves, possibly leading to increased transpiration. The consequences of this effect may be smaller heads. There is also a change in color, as neem-oil-treated cabbage plants become greenish instead of bluish-grey.

Smaller cabbage heads mean loss of potential yield, but the damage caused by unchecked DBM is in general much worse. In the Philippines it was much easier to sell the smaller heads from neem plots than the large heads from Bayer SIR 14591 plots (Table 6) on the market. No bitterness remained on neem-treated cabbage.

Table 6. Effect of neem extract and conventional insecticide treatments on cabbage yield and head damage by DBM (Kirsch 1986).

Insecticide, conc or applied amount	Yield (t/ha)	Head damage[a]
Control	34.6 a[b]	4.9 a[b]
Profenofos 500 EC, 0.05% AI	42.3 ab	4.9 a
BAYER SIR 14591 250 EC, 50 g AI/ha	71.1 c	1.0 d
Thuricide HP, 1 kg/ha	34.0 a	4.8 a
AZT-VR-K EC, 0.2%	52.9 b	2.6 b
AZT-VR-K EC, 0.2% + Thuricide HP, 1 kg/ha	47.3 ab	2.3 b
ANSKE, 25 g NSK/l	45.5 ab	1.5 c
ANSKE, 25 g NSK/l + Thuricide HP, ! kg/ha	44.3 ab	1.4 b

[a]Levels of damage: 1 = heads with no damage; 2 = heads with light damage, no trimming required; 3 = heads with light damage, trimming of one leaf required; 4 = heads with moderate damage, trimming of 2-3 leaves required; 5 = heads with severe damage, trimming of more than three leaves required.

[b]Within columns, means followed by a common letter are not signifiantly different at P = 0.05 (Duncan's Multiple Range Test).

ANSKE = Aqueous neem seed kernel extract, AZT-VR-K-EC = Formulated, methanolic neem seed kernel extract.

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Use of Benfuracarb in the Integrated Management of Diamondback Moth

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Abstract

Integrated pest management (IPM) is important in the control of diamondback moth, *Plutella xylostella* (L.), on brassicas in Japan. Recent investigations showed that benfuracarb 5% granules (5G) (trade name: Oncol 5G), a new carbamate insecticide, possesses useful properties in the IPM of diamondback moth. In order to obtain the best effect from benfuracarb 5G, application timing is very important. Pot tests and field trials indicated that application of 1-2 g of the granules in the hole or on the soil around the cabbage plants was effective up to 28 days after application. Effectiveness of benfuracarb 5G against susceptible and resistant strains of diamondback moth was determined using newly hatched larvae. Benfuracarb 5G applied to soil at a rate of 2 g per plant was highly effective against all strains on potted cabbage. Over 90% control was observed up to 28 days after application. Effect of benfuracarb 5G on the beneficial arthropods was also investigated. When 2 g of the granules were applied to the soil around cabbage and broccoli in the field, no adverse effect was observed on the population of spiders, important predators of diamondback moth.

Introduction

Diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), is a serious and widespread pest of many brassica crops, especially cabbage, and the development of resistance in this pest to a number of organophosphorus and carbamate insecticides has become a serious problem worldwide. Many researchers point out that integrated pest management (IPM) is an important way to control DBM. Recent investigations indicated that benfuracarb [ethyl *N*-[2,3-dihydro-2,2-dimethylbenzofuran-7-yloxycarbonyl (methyl) aminothio]-*N*-isopropyl-θ-alaninate], a new carbamate insecticide from Otsuka Chemical Co., Ltd. (Goto et al. 1983; Takagi 1989) possesses useful properties for the IPM of DBM. Benfuracarb 5% granular formulation (5G) is now widely used in Japan for the control of DBM.

This report describes our recent studies indicating the useful properties of benfuracarb 5G in the IPM of DBM.

Materials and Methods

Insecticides

The following insecticides were used throughout the study: benfuracarb (98.5% pure) and benfuracarb 20EC; acephate (>98%, Wako Pure Chemical Industries Ltd.); acephate 50WP (Takeda Chemical Industries Ltd.); permethrin (92.8%) and permethrin 20EC (Sankei Chemical Co., Ltd.); fenvalerate: malathion 40WP (Nihon Nohyaku Co., Ltd.); methomyl 45WP (Shell Chemical Co., Ltd.); chlorfluazuron 5EC (Sankyo Co., Ltd.).

Insects

Susceptible strains (S), resistant strains (R) and highly resistant strains (HR) of DBM were used. The S strain was provided in 1988 by Dr. H. Hama, Chugoku National Agricultural Experiment Station. The R strain was collected in 1989 and 1990 from a cabbage field of Otsuka Chemical Co. Ltd. in Tokushima Prefecture. The HR strain was collected in 1990 from the cabbage field of Mizobe-cho, Kagoshima Prefecture, Japan, where DBM populations have developed resistance to pyrethroid insecticides. The S strain was reared for several successive generations. The R and HR strains were reared for three to four generations in the laboratory at 25±1°C, photoperiod 16L:8D, by the method by Kaoshihara and Yamada (1976) with slight modification.

Susceptibility of DBM to benfuracarb and other insecticides

Susceptibility to benfuracarb and other insecticides was determined by the leaf-dipping method. Excised cabbage leaves, about 4 × 4 cm, were dipped for 1 min in aqueous solution containing a spreader (64 ppm) and each chemical. The treated and untreated leaves were put in a plastic cup (diam 8 cm, depth 4 cm) where 10 3rd instar larvae of DBM were released. The insects were maintained at 25±1°C, photoperiod 16L:8D for 48 hours. There were six replications per concentration of each insecticide.

Relation between leaf position and efficacy against DBM

Benfuracarb was applied onto the soil around the cabbage plant at four true leaf stage in Wagner pots at a rate of 1 or 2 g/plant. Each cabbage leaf was excised and put in plastic cups (diam 12 cm, depth 6 cm), where 10 first instar larvae of R strain DBM were released. The cups were maintained at 25±1°C, photoperiod 16L:8D, for 48 hours. Mortality of larvae was recorded 2 days after the release. Cabbage leaves were separated into three positions, i.e. upper, middle and lower positions. The test had six replicates.

Application timing of benfuracarb

Benfuracarb 5G (1 or 2 g/plant) was applied when cabbage plants had 3-4 leaves (in nursery bed) at 7 days before transplanting, at transplanting (4 leaf stage) and 7 days (5-6 leaf stage) or 14 days (8-9 leaf stage) after transplanting in 16 cm diameter Wagner pots. As a control, acephate 5G was applied at a rate of 2 g/plant. Cabbage leaves were excised at predetermined intervals (3, 7, 14, 21 or 28 days after transplanting) and placed in plastic cups where 10 hatched larvae of DBM (R strain) were released. The cups were maintained at 25±1°C, photoperiod 16L:8D, for 48 hours. Testing was conducted at six replications for each rate. In the case of application at transplanting, third instar larvae of R strain DBM were used for the evaluation of insecticidal activity of benfuracarb.

Efficacy of benfuracarb against three different strains of DBM

Benfuracarb 5G (2g/plant) was applied on the soil around cabbage plants at transplanting from the vinyl pots to 16 cm Wagner pots. Cabbage leaves were excised 3, 7, 14, 21 and 28 days after the transplanting and placed in the plastic cups where 10 hatched larvae of DBM were released. The cups were maintained in the laboratory at 25±1°C, photoperiod 16L:8D, for 48 hours. There were six replications for each rate. Acephate 5G was used as a control at a rate of 2 g/plant.

Field trial of benfuracarb in cabbage

The field trial for the efficacy of benfuracarb 5G against DBM, green peach aphid and common cabbage worm was conducted at our experimental farm in Tokushima Prefecture. Benfuracarb 5G (1 or 2 g/plant) was applied to the soil around the cabbage plants (Shikidori) at 5-6 leaf stage on 5 June 1990 on three 20-m rows (spacing 0.5 m). Acephate 5G was used as a control at a rate of 2 g/plant. The number of pest insects on 20 cabbage plants in each row was counted.

Efficacy of benfuracarb on spiders

To study the effect of benfuracarb 5G on the population of spiders one experiment each was conducted in a cabbage field (30 m × 2 rows) in Hiketa-cho, Kagawa Prefecture, Japan, and in a broccoli field (25 m × 2 rows) in Ichiba-cho, Tokushima Prefecture, Japan. Benfuracarb 5G (2 g/plant) was applied to the soil around cabbage plants (Kogetsu) of 6-7 leaf stage on 8 September 1990 and broccoli plant (Haitsu) of 5-6 leaf stage on 7 September 1990. The number of spiders on 50 plants in each row was counted 5, 12, 19 and 27 days after the application to cabbage, and 7, 15, 22 and 32 days after the application to broccoli. In the conventional application plots methomyl 45 WP was applied to cabbage on 23 September 1990 and fenvalerate: malathion 40WP, chlorfluazuron 5EC and permethrin 20EC were applied to broccoli on 15 and 24 September and 1 October, respectively.

Results

Comparison of susceptibility of DBM larvae to benfuracarb and other insecticides

The LC_{50} values of benfuracarb and other insecticides for three different strains of DBM larvae are shown in Table 1. The LC_{50} of benfuracarb in the 1990 testing was 14, 48 and 573 ppm for S, R and HR strains, respectively. The resistance ratio was 3.4 in the R strain and 40.9 in the HR strain. The data indicated similar susceptibility of both S and R strains in 1989 and 1990, while HR strain showed high levels of resistance.

In contrast to this, both R and HR strains showed quite high levels of resistance to other insecticides. The level of resistance to insecticides was extremely high in the HR strain.

Table 1. Susceptibility of 3rd instar DBM larvae to benfuracarb (leaf dipping method).

Insecticides		LC_{50} (ppm)		
		S strain[c]	R strain[d]	HR strain[e]
Benfuracarb	Technical[a]	9	24	–
	Formulation[b] (20 EC)	14	48	573
Methomyl	Formulation (45 WP)	145	613	1579
Acephate	Technical	8	173	–
	Formulation (50 WP)	5	97	3984
Permethrin	Technical	9	73	–
	Formulation (20 EC)	5	44	364
Fenvalerate (10%) Malathion (30%)	Formulation (40 WP)	26	359	3943

[a]Trial in 1989; [b]Trial in 1990; [c]Susceptible strain; [d]Resistant strain; [e]Highly resistant strain.

Relation between leaf position and efficacy against DBM

Benfuracarb 5G at a rate of 2 g/plant showed 100% mortality in all leaf positions 10 days after treatment (Table 2). After 20 days 100% mortality was observed in middle and lower leaf position, while the mortality was lower (about 80%) in upper leaf position. The same tendency was observed at 1 g/plant.

Table 2. Efficacy of benfuracarb 5G against 1st instar DBM larvae (R strain) fed on cabbage leaf of different positions.

Leaf position	Mortality (%) at indicated days after treatment			
	10 days		20 days	
	1 g/plant	2 g/plant	1 g/plant	2 g/plant
Upper	91	100	54	81
Middle	100	100	100	100
Lower	100	100	100	100

Application timing of benfuracarb

Application timing is very important to obtain the highest level of effectiveness of benfuracarb 5G against DBM (Fig. 1-3).

The best effect was obtained by application of granules at transplanting. Complete mortality was obtained up to 28 days after the application even at a rate of 1 g/plant. Slightly lower efficacy was observed with other application timings. Benfuracarb 5G was also effective against 3rd instar larvae when applied at transplanting (Fig. 3).

Efficacy of benfuracarb against three strains of DBM

Insecticidal activity of benfuracarb against S, R and HR strains was determined using hatched larvae and excised cabbage leaves of middle leaf position from the potted cabbage plants which were treated with benfuracarb 5G. As shown in Fig. 4, benfuracarb 5G was highly effective against all three strains of larvae by plant foot application at a rate of 2 g/plant. Over 90% control was observed from 7 to 28 days after the application. Acephate 5G used as a control was less effective and lacks residual activity especially against the HR strain.

Field trial for benfuracarb in cabbage

A field trial was conducted in 1990 in order to confirm the effectiveness and residual activity of benfuracarb 5G which was observed in the potted plant test. In the field trial efficacy of benfuracarb 5G was determined not only for DBM, but also for green peach aphid and common cabbage worm because of the occurrence of the three pests in the the field trial. As shown in Fig. 5, effectiveness of benfuracarb 5G against all three pests was observed 28 days after the treatment at a rate of 1 or 2 g/plant.

Effect of benfuracarb on spiders

In order to see the effect of benfuracarb 5G on the beneficial arthropods, field trials were conducted in 1990 on cabbage and broccoli. The effect of benfuracarb 5G on the population of spiders in each field was determined following plant foot application of the granules (2 g/plant). Benfuracarb 5G did not have any effect on the population of spiders, important predators of DBM, in cabbage and broccoli fields. Mean number of spiders per plant in both

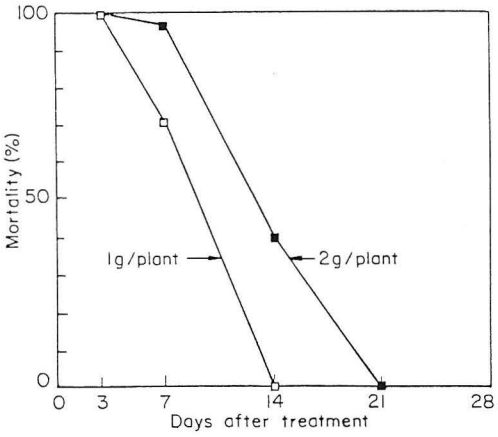

Fig. 1. Efficacy of benfuracarb 5G against 1st instar larvae (R strain) of DBM on potted cabbage treated 7 days before transplanting in nursery bed.

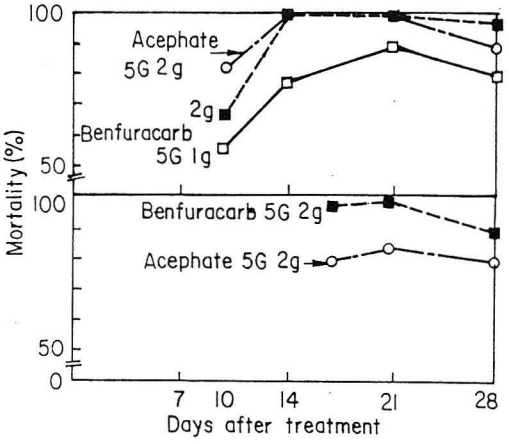

Fig. 2. Efficacy of benfuracarb 5G against 1st instar larvae (R strain) of DBM on potted cabbage treated 7 days after transplanting (above) and 14 days after transplanting (below).

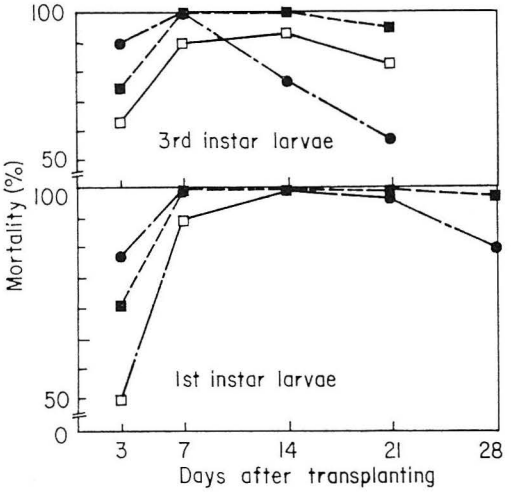

Fig. 3. Efficacy of benfuracarb 5G treated at transplanting against DBM larvae (R strain) on potted cabbage. (■) benfuracarb 5G 2g, (□) benfuracarb 5G 1g, (●) acephate 5G 2g a) Days after transplanting.

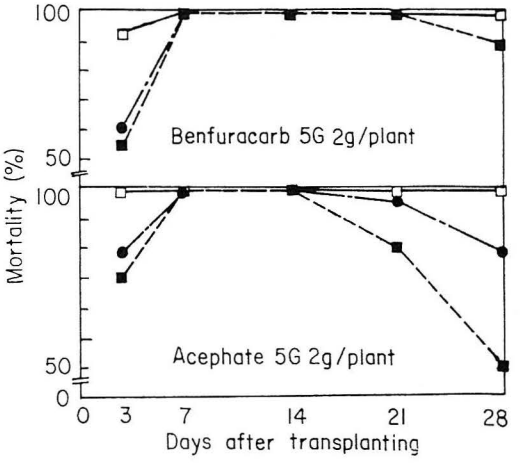

Fig. 4. Efficacy of benfuracarb 5G and acephate 5G treated at transplanting against 1st instar larvae of DBM strains on potted cabbage. (□) S strain; (●) R strain; (■) HR strain.

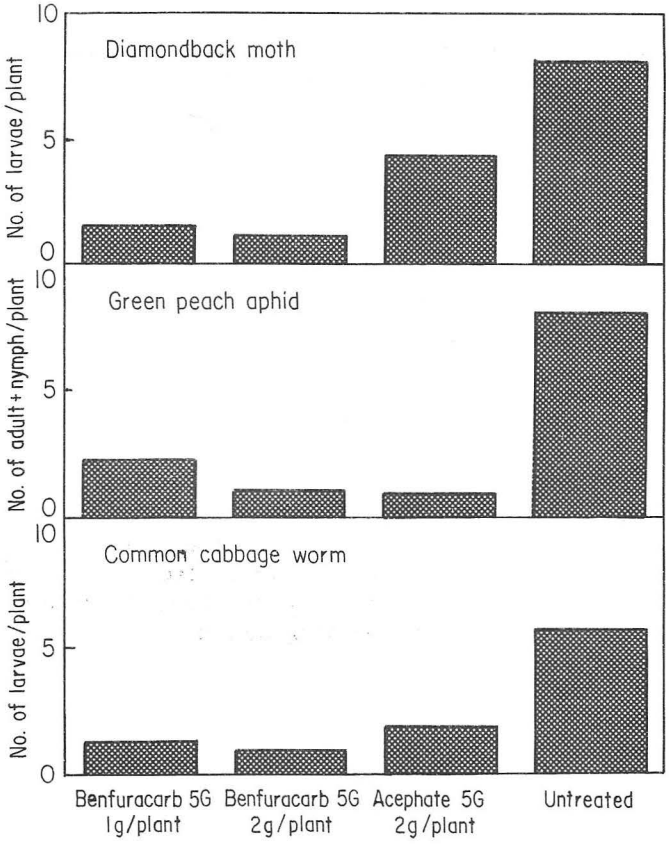

Fig. 5.
Efficacy of benfuracarb 5G against cabbage pests in the field trial. Observations were made 28 days after transplanting.

benfuracarb 5G-treated and untreated plots increased from 1 to 2 during the 1-month test period. On the other hand, populations of spiders decreased significantly in the plots where methomyl or synthetic pyrethroids were sprayed.

Discussion

Development of pyrethroid resistance in DBM in Japan was first confirmed in Kagoshima, Miyazaki and Okinawa prefectures in 1984 (Koshihara 1988), and existence of resistance was reported all over Japan including Hokkaido by the summer of 1990. Development of cross-resistance among pyrethroid insecticides (Hama 1987) in addition to cross-resistance among organophosphorus insecticides (Hama 1986) has become a serious problem for growers in controlling DBM by insecticides.

Current studies indicated that benfuracarb, a new carbamate insecticide, is effective in controlling DBM, even R and HR strains when granular formulation (5G) is applied to the soil. The systemic activity of benfuracarb 5G is probably responsible for insecticidal activity and residual effectiveness. The effectiveness of benfuracarb 5G against the HR strain, one of the most resistant strains in Japan (Horikiri and Makino 1987), was also confirmed in the field trial by Tanaka et al. (1990).

It is very important that an insecticide used in IPM is not toxic to predators. The results indicated that benfuracarb 5G does not affect the population of spiders — in cabbage and broccoli

fields following soil treatment. In the same field trial, the population of spiders was significantly reduced by conventional use of methomyl or pyrethroid insecticides. Nemoto (1986) reported that application of methomyl 45WP caused reduction in the population of the spider *Pardosa astrigera*.

Benfuracarb 5G thus possesses useful properties for the IPM of DBM. A number of trials have been conducted by researchers in Japan using benfuracarb 5G in an IPM program, i.e. a combination with foliar insecticides, protective net or DBM sex pheromone. Horikiri (1989) proposed a reduction in the number of applications of conventional insecticides by combined use of benfuracarb 5G and other insecticides.

Morishita and Azuma (1990) reported the usefulness of combined use of benfuracarb with nets in DBM control programs in cabbage fields. Iwata (1989) also studied the use of DBM sex pheromone with two applications of benfuracarb at transplanting and conventional insecticides (one time) 30-40 days after the transplanting, instead of five applications of conventional insecticides, which provided excellent control of DBM. It would be of interest to consider using benfuracarb 5G in combination with *Trichogramma chilonis*, an egg parasite of DBM (Iga 1985), in a DBM control program.

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Developing A Reduced Spray Program for Brassicas in New Zealand

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Abstract

Control of lepidopterous pests on vegetable brassicas in New Zealand is largely reliant on fortnightly applications of synthetic pyrethroid insecticides. Both diamondback moth (*Plutella xylostella* (L.)) and white butterfly (*Pieris rapae* (L.)) are serious pests. Although insecticides still give effective control of these pests, diamondback moth has recently been reported to be tolerant to some insecticides in New Zealand. Research was initiated in an effort to reduce insecticide use and delay the development of insecticide resistance. This program included a reevaluation of the previously introduced ichneumonid parasitoids, *Diadegma semiclausum* Hellen and *Diadromus collaris* (Gravenhorst), which indicated that these species are poorly synchronized with their host. Importation of further natural enemies is proposed. A reduced spray program, based on a scouting technique which uses the percent of plants infested to estimate pest populations, is also being developed. Use of thresholds in experimental trials reduced insecticide usage by 25-50%. An action threshold of 15% infested plants resulted in less damage than fortnightly applications. Implementation trials are presently aimed at training growers to use this system to make rational spray decisions.

Introduction

Two thousand eight hundred hectares of vegetable brassicas are grown each year in New Zealand; cabbage and cauliflower each account for 36% of the area, broccoli 21% and brussels-sprouts 7%. Vegetable brassica production occurs throughout the country. In the subtropical north (latitude 35°S) most lepidopterous pests of brassicas are active year-round, whereas in the south (46°S) average winter temperatures below 5°C minimize activity. The crop is grown primarily near major population centers as the majority of brassica crops are produced for domestic consumption. Our studies are located at Pukekohe, in the Auckland area, which produces 26% of New Zealand's brassicas. Pukekohe produces a variety of crops besides brassicas, including potatoes, onions, and squash. Brassicas are mostly used for the fresh market where quality is very important, and minimal damage is accepted.

Brassica growers at Pukekohe plant an average of 15 ha in vegetable brassicas and most properties are run within the extended family. The mixed vegetable plantings are surrounded by pasture, glasshouse production, and field crops. Cabbage is grown throughout the year, while broccoli and cauliflower are mostly produced in spring and summer. Brassicas are planted in 0.5-1 ha strips in sequential plantings usually 10 days apart; the most common mixture is side-by-side plantings of cabbage/broccoli or cabbage/cauliflower. Current practice for insect control revolves around calendar insecticide applications, as growers do not have specialized training necessary to identify pest infestations.

We identified two areas which could improve control and decrease insecticide use: first, the evaluation of existing lepidopterous pest levels and levels of natural control to determine the

need for further importations of parasitoids; and second, the development of a pest monitoring system which could allow growers to identify damaging pest populations and reduce the need for regularly scheduled insecticide applications.

Insecticide Use Patterns

Pukekohe brassica growers apply insecticide to control diamondback moth (DBM) *Plutella xylostella* (L.), (Lepidoptera: Yponomeutidae) and white butterfly (WB) *Pieris rapae* (L.), (Lepidoptera: Pieridae) at 10-14-day intervals. This interval increases in the winter and decreases in the summer when the pressure from pests is greatest. A variety of insecticides is used but synthetic pyrethroids are most commonly applied. Insecticide is generally applied as a preventive spray, regardless of plant varieties, plant ages, or infestation levels. Growers do not scout for infestations and farms are too small to make the use of private consultants economically feasible.

In 1987 the New Zealand Committee on Pesticide Resistance identified DBM as a serious potential resistance problem. Overseas, DBM has shown the ability to develop resistance to all major groups of insecticides (Sun et al. 1986). No insecticide failures have been reported in New Zealand crops, but tolerance to esfenvalerate, permethrin, diazinon, mevinphos, and carbaryl have recently been reported in populations collected from Pukekohe (Bell and Fenemore 1990). Public concern over the presence of chemical residues in food is increasing and some residue testing by supermarket chains has been undertaken, but the results are not yet known.

Pest Management Options

Brassica growers operate their farms as family operations and usually there is no specialized knowledge of pest management. In a survey, growers identified their greatest concern with initiating a scouting program to be the time required, rather than the cost of unnecessary sprays. At the same time, as residue testing becomes more commonplace, grower concern over excessive use of insecticides is increasing. There is no existing government extension service for fresh market producers, and private sector consulting services are rare. To enable growers to break their reliance on calendar scheduled applications, the options presented to them must be both quick and simple (Wearing 1988). Two options which we felt would fit these requirements were the improvement of biological control and use of a reduced spray program.

Biological control agents were imported in the 1930s and had not been extensively evaluated since the 1950s (Todd 1959). The possibility exists that new natural enemies could be introduced to complement existing biological control.

The development of a reduced spray program which would be attractive to New Zealand brassica growers involves several key constraints. Scouting should be minimized, so that the time required to reach a no-spray decision is less than the time required to spray that same field. A conservative action threshold is also necessary, as stringent market requirements demand near-perfect produce. Finally, a reduced spray program must eventually include broccoli and cauliflower to suit the farming practices of Pukekohe growers.

Status of Lepidopterous Pests and their Parasitoids

Background

DBM is an introduced species in New Zealand where in most areas it is active throughout the year. Muggeridge (1930) considered it to be the most serious pest of brassica crops. As existing parasitoids provided inadequate control Muggeridge (1930) proposed the introduction of new species. This commenced in 1936 (Thomas and Ferguson 1989), and resulted in the

successful establishment of the larval parasitoid *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae) and the pupal parasitoid *Diadromus collaris* (Gravenhorst) (Hymenoptera: Ichneumonidae). *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae) was imported but not released. Subsequent evaluation of pest status and parasitism from 1955 to 1958 (Todd 1959) indicated a high degree of control of DBM in fodder crops, but insufficient impact of parasitoids to provide acceptable quality in high-value vegetable crops. *Diadegma semiclausum* was the most effective of the two parasitoids. Reports of this success led to the export of both species to Australia, Malaysia and the Cook Islands, and *D. semiclausum* to Indonesia (Thomas and Ferguson 1989).

WB was first reported in 1930 (Muggeridge 1942), and within 6 years it had spread throughout both the North and South Islands, achieving pest status on forage and vegetable brassica crops. Knowledge of the control exerted by parasitoids and disease overseas led to the initiation in 1931 of a parasitoid introduction program (Muggeridge 1943). The pupal parasitoid *Pteromalus puparum* (L.) (Hymenoptera: Pteromalidae) was established in 1933, and by 1934 89% of host pupae were parasitized over a wide range of sites. In a later evaluation of WB in fodder brassica crops, Todd (1959) showed that larval populations were reported to be low and causing little damage, while pupal parasitism remained at high levels. The larval parasitoid *Apanteles glomeratus* (L.) (Hymenoptera: Braconidae) was widely established by 1959 (Ferguson 1989). In combination with a granulosis virus, *A. glomeratus* can cause significant larval mortality, but Ashby and Pottinger (1974) confirm that pupal mortality, particularly that caused by *P. puparum*, is most important in determining population trends.

Less serious lepidopterous pests regularly causing damage to brassicas are the soybean looper (SBL) *Thysanoplusia orichalcea* (F.), a recent immigrant from Australia (Hill et al. 1987), and *Helicoverpa armigera* (Hübner) which is less common.

Current pest status and biological control

The present study includes a reevaluation of brassica pests and their natural control in vegetable brassicas. This has been carried out in a sequence of unsprayed cabbage plots planted at the DSIR Pukekohe Research Station. Routine sampling and laboratory examination provided population counts of all stages and species of larvae (except 1st instar DBM in mines) and pupae. All material, including random collections of eggs, was reared to determine its fate. The average population density per cabbage for each of six successive crops is shown in Fig. 1. For the two years shown DBM was the most common species in spring or early summer, whereas WB reached a peak in summer or autumn. Natural populations of DBM on vegetable brassicas can reach high levels in New Zealand, with over 100 3rd and 4th instar larvae per plant in some spring crop samples. For all four spring and summer crops unprotected plants sustained severe damage. Soybean looper caused damage only in late summer, but as these larvae fed only on the under-surface of outer wrapper leaves this damage was less economically important than that caused by DBM or WB. Winter populations of all species were negligible, as were populations of *H. armigera* in all crops.

The presence of all four introduced parasitoids of DBM and WB was confirmed, as was the absence of egg parasitoids. In spring 1988, parasitism of DBM was at a peak of 85% early in the crop (Fig. 2). This was totally due to *D. semiclausum*, but failure of this parasitoid to respond to the increasing pest population allowed DBM pupae to reach over 60/plant. By contrast, *D. collaris* exhibited a delayed response and reached a level of 35% parasitism late in population development in this crop (Fig. 2). A hyperparasitoid, *Trichomalopsis* sp. (Hymenoptera: Pteromalidae), may have accounted for a late decline in parasitism by *D. semiclausum*. The seasonal variation in DBM parasitism (Table 1) indicated that *D. semiclausum* was active year-round but that there was little relationship between levels of parasitism (Table 1) and host density (Fig. 1). Similar results were seen in WB parasitized by *A. glomeratus*. Infestations early in particular crops were heavily parasitized, but as pest populations increased parasitism declined. *A. glomeratus* was more active in summer and autumn crops (Table 1).

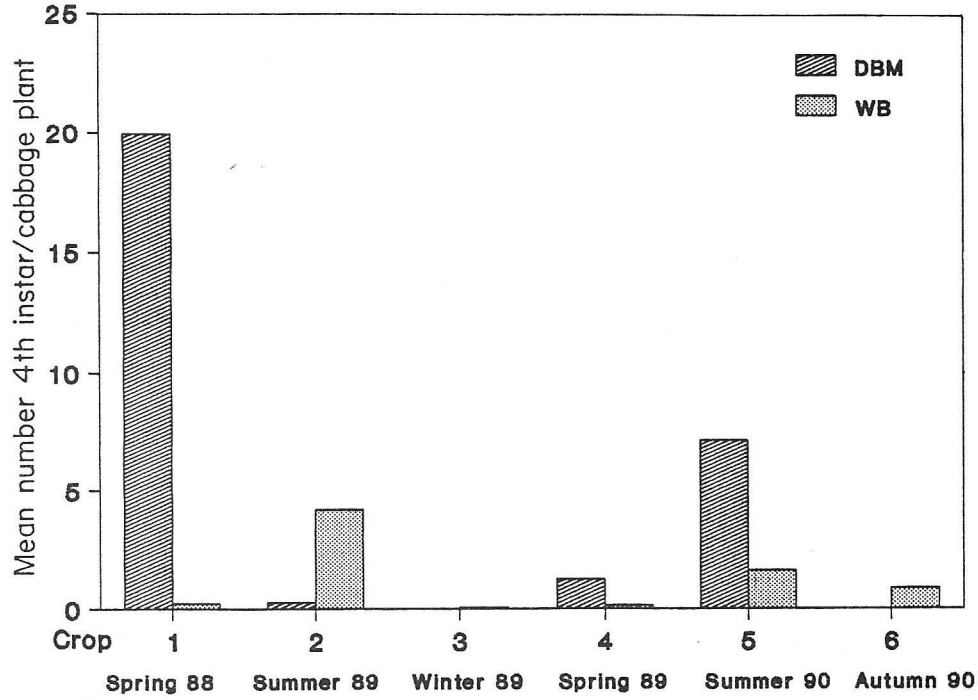

Fig. 1. Seasonal comparison of DBM and WB fourth instar larval populations in unsprayed cabbage plots in Pukekohe.

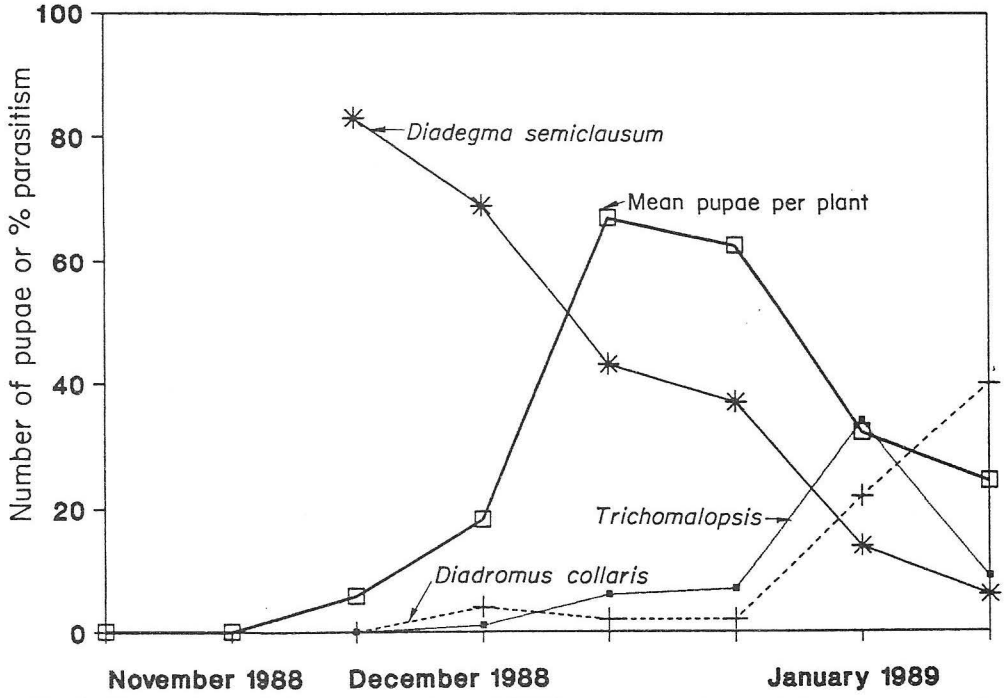

Fig. 2. Comparison of the mean number of DBM pupae per plant and the percentage of those pupae parasitized by three parasitoids during spring 1988 in unsprayed cabbage plots in Pukekohe.

Table 1. Percent parasitism determined from DBM pupae and WB 5th instar larvae and pupae collected from unsprayed plots in Pukekohe.

A. DBM pupae.

Crop	% Ds	Range	% Dc	Range	% Tr	Range	N
1[a]	36	6-83	11	0-40	10	0-34	1267
2	10	0-100	0	0	0	0	27
3	33	0-57	0	0	0	0	12
4	63	49-74	2	0-8	0	0	190
5	31	13-85	13	0-36	7	0-18	570
6	47	43-67	10	0-50	0	0	21

Ds = *Diadegma semiclausum*, Dc = *Diadromus collaris*, Tr = *Trichomalopsis spp.*
[a]Refer to Fig. 1.

B. WB 5th instar larvae and pupae.

Crop	Larvae			Pupae		
	% Ag	Range	N	% Pp	Range	N
1	0	0	7	0	0	3
2	41	0-65	370	5	0-33	19
3	20	0-100	10	0	0	3
4	20	0-100	15	10	0-25	10
5	48	0-58	172	8	0-14	59
6	81	43-100	58	0	0	1

Ag = *Apanteles glomeratus*, Pp = *Pteromalus puparum.*

The current study has shown parasitism of DBM at levels comparable to early evaluations (Todd 1959). Parasitism of WB was considerably lower than studies by Todd (1959) on fodder crops but similar to that observed by Ashby and Pottinger (1974) in vegetable brassicas. These results could suggest some suppression of parasitoid activity by wide use of insecticides in vegetable-growing areas. It is clear that the parasitoids are not well synchronized with their hosts and that additional species that attack and kill the early larval stages could provide better protection.

Reduced Spray Program

A reduced spray program must fulfil two basic requirements if it is to be successfully implemented by New Zealand brassica growers. First, the scouting method must be simple, quick, and effective in determining infestation levels. Second, the action threshold must maintain produce quality while still decreasing the frequency of applications.

IPM scouting methods in cabbage can be divided into three categories (Cartwright et al. 1987): density counts of eggs and/or larvae, percent of plants infested, and damage counts. We tested various action threshold levels which allowed a range of infestation levels to develop. We also assessed two existing scouting methods, comparing larval density counts and percent of plants infested. Counts of damage were not considered practical given the stringent market requirements.

In all trials, treatments were compared to an unsprayed control and to a grower standard (i.e., fortnightly calendar application). Each succeeding trial incorporated refinements based on results of the prior trial.

Scouting methods were compared at the DSIR Mount Albert Research Centre in Auckland, while other trials were carried out at the DSIR Pukekohe Research Station. All trials used a randomized complete block layout with a minimum plot size of 36 plants. When a treatment reached its threshold, permethrin was applied at the rate of 0.05 kg AI/ha. Weekly sampling continued until harvest when damage was assessed as follows: outer wrapper leaves (score 1-3);

inner wrapper leaves (score 1-3); head (score 1-6); and all heads were weighed. A cumulative score of 3 identified a perfect, undamaged cabbage; a cumulative score of >7 indicated an unmarketable cabbage. The severe damage observed in all control plots (Table 2) showed that pests were abundant. Analyses were made on SAS (SAS 1985) using LSD ($P<0.05$).

Table 2. Comparison of scouting methods and action thresholds based on harvest damage totals, head weight, number of applications, and percent of premium (P) (damage score = 3 to 4) and acceptable (A) (damage score = 3 to 6) heads of cabbage per treatment.

Treatment	N	Damage Total[a]	Head Wt (kg)	Number of appl.	%P	% A
a. Action Threshold Comparison						
Standard	48	4.80 d[b]	2.19 a	5	45	86
Low (0.25 FDE)	48	5.04 d	2.18 a	3	43	86
Med (0.5 FDE)	44	7.00 c	1.98 ab	3	2	43
High (1.0 FDE)	51	7.92 b	1.88 bc	1	0	15
Control	39	9.95 a	1.58 c	0	0	0
b. Scouting Method Comparison						
Standard	44	3.18 e	1.96 a	4	100	100
20% infested	57	4.98 d	1.84 ab	2	40	93
0.5 CLE	58	6.43 b	1.87 ab	1	9	57
0.5 FDE	61	5.62 c	1.83 ab	2	23	77
Control	46	9.33 a	1.75 b	0	0	0
c. 15% vs 20% Infested Plants Threshold						
Standard	80	6.42 c	1.42 b	4	6	54
15% infested	80	5.88 d	1.52 a	3	14	78
20% infested	80	7.68 b	1.46 ab	4	5	15
Low (0.25 FDE)	80	7.80 b	1.45 ab	2	0	22
Control	80	11.90 a	1.31 c	0	0	0
d. 15% Infested Threshold						
Standard	120	4.97 b	2.29 b	5	36	77
15% infested	120	4.85 b	2.43 a	7	52	87
Control	120	10.01 a	1.66 c	0	0	0

[a]Damage score (outer + inner wrapper leaves + head) ranges from 3 (perfect) to 12 (completely unusable). [b]Means followed by different letters are significantly different ($P<0.05$).

Action threshold comparison

To determine levels of lepidopterous pest infestations cabbage could sustain before unacceptable damage occurred, we compared the impact of three levels of pest populations. Population estimates were based on larval density per plant. Our sampling was based on feeding damage equivalents (FDE), a modification of the cabbage looper equivalents (CLE) of Shelton et al. (1982, 1983), which recognized that different sized larvae produced different amounts of damage. The values we used were: 1 DBM = 0.25 FDE. Noctuid or pierid larvae were counted as: 3rd instar = 0.5 FDE, 4th instar = 1.0 FDE, 5th instar = 1.5 FDE. This gives more importance to DBM than did Shelton et al. (1982, 1983). The thresholds used were low, medium, and high larval populations (0.25, 0.5, and 1.0 FDE/plant, respectively).

The results (Table 2a) show that there was no significant difference in total damage, head weight, or percent of plants graded acceptable between the standard and the 0.25 FDE action threshold, although the low threshold plot received two fewer applications of insecticide. Levels of damage were unacceptable in the remaining treatments.

Scouting strategy comparison

'Control' and 'standard' treatments were compared to (1) percent infested plant method, using the action threshold of 20% of plants infested (Theunissen and den Ouden 1985); (2) larval density method of Shelton et al. (1982, 1983) based on cabbage looper equivalents (CLE) at their action threshold of 0.5 CLE/plant; and (3) the larval density modification of feeding damage equivalents (FDE see above) at the action threshold of 0.5 FDE/plant (i.e., medium larval density).

The results (Table 2b) showed that least damage occurred in the standard treatment. The most promising treatment, in terms of reliably assessing damaging population levels, was the '20% infested' treatment. This treatment produced only 7% fewer acceptable cabbages while receiving two fewer insecticide applications than the 'standard'. The time required to scout 40 plants in the '% infested,' 'CLE,' and 'FDE' treatments when no infestation was present was 20 min for each treatment. As infestation levels increased, the time spent scouting in the '% infested' plots decreased to a minimum of 8 min. The opposite occurred in scouting larval density using 'CLE' or 'FDE' counts -- the greater the infestation, the more time required to assess it, up to a maximum of 60 min.

This trial showed that counting the percent of plants infested fulfilled the requirements of being simple, fast, and easy to calculate. An action threshold of 20% infested plants was too liberal and resulted in a decrease of the percent of plants classified as 'premium.' If the data from the 0.25 FDE treatment from the previous trial comparing thresholds was converted to percent of plants infested, it appeared that the '0.25' FDE action threshold was roughly equivalent to 15-35% of the plants infested (Beck and Cameron 1990a). We refined this treatment in the next season by lowering this action threshold to 15% infested plants.

15 vs 20% vs 0.25 FDE thresholds

In this trial we modified slightly our sampling procedure. In earlier trials we observed that a field infestation of 12-14% could increase to 60% within a week. We retained the '15% infested' threshold with the modification that if a '12-14% infestation' was observed, the sample was repeated in 3 days. The '20% infested action' threshold was not changed, nor was the '0.25 FDE' threshold.

The '15% infested' threshold in this trial resulted in less damage (Table 2c) than other treatments. When compared to the 'standard,' the '15% infested' treatment produced 24% more acceptable cabbages with one less spray. Insect pest pressure in this trial was extremely high; the mean damage total for unsprayed cabbages was 11.90 out of a possible high score of 12.00. The timing of applications was crucial. The first two sprays were applied within a short interval in the '15% infested' treatment; the infestation level remained high at 52.5% (Fig. 3) after the first application, as DBM eggs continued to hatch. The second application reduced infestation levels to 10% infested. The 'standard' treatment was not sprayed until the following week (Fig. 3). The '20% infested' and '0.25 FDE' treatments allowed unacceptable damage to occur (Table 2c).

15% infested threshold

In the final trial we compared the '15% infested' action threshold (with repeat sampling for infestations between 12 and 14%) to 'standard' and unsprayed 'control' treatments. In the summer of 1990, oviposition pressure by all three lepidopterous pests was high and continuous. The action threshold treatment (Table 2d) produced 10% more acceptable cabbages than the standard, but also required two more spray applications to achieve this.

We are applying the results from small field plots to commercial fields this coming season. Growers are interested in reducing insecticide input without decreasing quality or yield. Their commitment to this approach will now be tested by their willingness to implement recommendations.

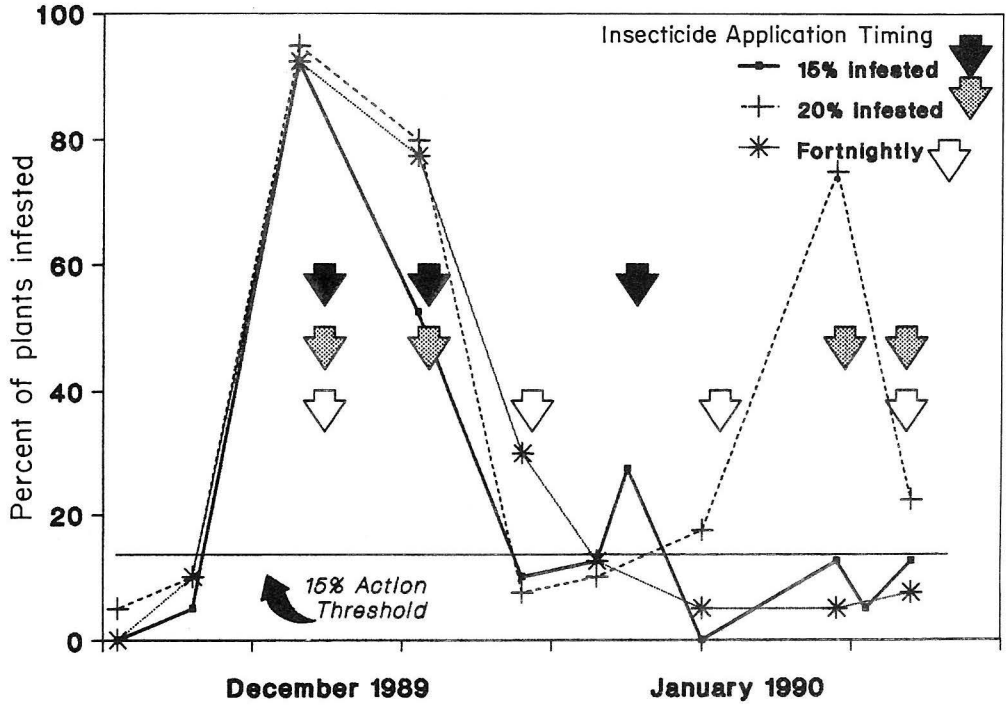

Fig. 3. The percent of plants infested in cabbage treated at two action thresholds compared with calendar scheduled application in summer 1990 in Pukekohe.

Other Vegetable Brassicas

When vegetable brassica cultivars are planted in adjacent strips, normal grower practice is to apply pesticides to all plantings regardless of plant cultivar or age. We questioned this practice, as little is known about the relative attractiveness of cabbage, broccoli, and cauliflower to either DBM or WB, and current research indicates that early season applications to broccoli (Vail et al. 1989) and cauliflower (Stewart and Sears 1988) may be unnecessary. In spring 1989 we compared population levels on cabbage, broccoli and cauliflower by taking weekly whole-plant larval density counts through one growing season at two locations (Beck and Cameron 1990b). The three cultivars had similar populations of small DBM larvae, suggesting no oviposition preference. Differences were seen in rates of parasitism (highest in broccoli) and in 4th instar and pupal populations (lowest in broccoli). Differences were also seen in larval location on the plants. More than 50% of the DBM larvae moved into the cabbage terminal by week 5; this occurred by week 7 in cauliflower, and did not occur in broccoli (Beck and Cameron 1990b). Florets were first observed in broccoli and cauliflower 8 weeks after transplanting; at this stage, a single application of permethrin effectively controlled existing pest populations.

We are continuing trials comparing pest populations in cabbage, broccoli, and cauliflower, to see if these observations apply in seasons when WB is dominant. Additionally, we are examining the possibility of producing marketable crops when insecticides are applied only after floret production.

Conclusions

1. *Diadegma semiclausum* is the major parasitoid of DBM but does not give sufficient control in vegetable brassicas. The effectiveness of *Pteromalus puparum* against WB may have

declined since its establishment. A study of the phenology of these pests and associated parasitoids indicates that further importations could enhance the existing natural control.
2. The scouting method of counting the percentage of plants that are infested is shown to suit the requirements of New Zealand brassica growers -- it is simple, fast, and effective.
3. An action threshold of 15-20% infested plants gave good yields in three trials, and under most conditions decreased insecticide applications by comparison with a fortnightly schedule.
4. Preliminary work comparing infestation levels in cabbage, broccoli and cauliflower showed that survival of DBM is lower on broccoli and cauliflower. Additionally, our observations and the literature suggest early season applications of insecticide may not be necessary prior to floret production in broccoli and cauliflower.
5. We are currently extending this scouting and action threshold of 15% infested plants to commercial fields.

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Economics of Managing Lepidopterous Cabbage Pests In the Southwestern United States

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Abstract

Studies were conducted in 1989 and 1990 at Lane, Oklahoma, to evaluate the economic benefits of managing lepidopterous pests of fresh-market cabbage using *Bacillus thuringiensis* Berliner var. *kurstaki*, or a pyrethroid insecticide in combination with an economic threshold based on larval density. Marketable cabbage yield and percent head damage were not significantly different among plots sprayed weekly compared with those sprayed according to the economic threshold, regardless of the type of insecticide used. Among weekly and threshold-based spray programs with both types of insecticides, fewer sprays were required with a pyrethroid/threshold program whereas the most applications were needed in the weekly-scheduled regimes. When timing was based on the economic threshold, control with the biological insecticide required more sprays than that with the pyrethroid. The least insecticide cost and greatest net returns were achieved when a pyrethroid/threshold regime was followed. The biological/threshold program required more sprays because more time was needed to effect larval mortality and thus larval counts changed less rapidly. Data were obtained from pesticide applicator professionals and cabbage producers to determine the effects of diamondback moth, *Plutella xylostella* (L.), insecticide resistance on the economics of managing lepidopterous pests of cabbage in the southwestern United States. Total amounts of insecticides used, numbers of applications, and total costs of pest control programs increased substantially in two cabbage production areas of southwestern United States where diamondback moth control failures have been observed.

Introduction

Lepidopterous larvae are the most serious pests of fresh-market cabbage throughout much of the United States. In the southwestern USA, cabbage looper *Trichoplusia ni* Hübner (CL) (Lepidoptera:Noctuidae) and the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera:Yponomeutidae), are the most devastating of the lepidopterous pest complex (Cartwright et al. 1987). Until recently, CL has been the more common of the two, but in recent years DBM has become more problematic. Control of both pests has been achieved historically with a range of insecticides, however, DBM has recently shown resistance to synthetic organic insecticides in the Lower Rio Grande Valley of Texas (Magaro and Edelson 1990), and in the Texas High Plains (Morrison, unpublished data), but not in Oklahoma. Because of concern for insecticide resistance, increased use of biological insecticides has been recommended (Kahn et al. 1990). Use of *Bacillus thuringiensis* Berliner var. *kurstaki* has been shown to be effective

in reducing CL and DBM populations, although some delay in mortality is observed when compared with that of other insecticide classes.

Use of economic thresholds has proven to be a valuable tool in managing lepidopterous pests of cabbage (Green 1972; Shelton et al. 1983). In the southwestern USA, Kirby and Slosser (1984) developed a composite economic threshold for lepidopterous larvae on cabbage. Cartwright et al. (1987) further demonstrated that the composite threshold of 0.3 larva/plant, as proposed by Kirby and Slosser (1984), resulted in reduced insecticide applications when used on a commercial basis. The development of currently used thresholds in the Southwest has been based on using synthetic organic insecticides to reduce larval populations below threshold. Because biological insecticides work more slowly and moribund larvae are not easily recognized, population estimates of larvae in plots treated with *B. thuringiensis* are likely to be overestimated, relative to those treated with an insecticide such as permethrin, in which the activity occurs within a fairly short period. Therefore, it is important to determine if the established economic thresholds can operate effectively when biological insecticides are used.

Workman et al. (1980) investigated the use of a damage-based management threshold for CL and DBM in conjunction with three insecticide classes, however, no economic analysis was provided. Because the accepted threshold for lepidopteran pests is based on insect counts, it is important to evaluate the threshold in combination with different insecticide types. Of particular interest was whether or not the overestimation of larval counts in *B. thuringiensis* treated cabbage would result in greater insecticide use. Thus, the objective of our field studies was to compare the season-long effectiveness and the economic benefit of a biological insecticide with a pyrethroid insecticide when applied based on weekly schedules or using an established economic threshold.

Until recently, control of lepidopterous pests in the southwestern USA has relied primarily on the use of organophosphosphorus, carbamate and pyrethroid insecticides. However, problems with controlling DBM have altered insecticide use patterns and had an enormous impact on cabbage production. DBM control failures were first noted in 1987 in the two major cabbage production areas of Texas, the Lower Rio Grande Valley and the High Plains, and became widespread by 1988. Once resistance became apparent, dramatic changes in the insect control programs became necessary. As part of evaluating the economics of controlling lepidopterous pests in the southwestern United States, we report on these changes and highlight the economic impact that DBM resistance has had on producers in the region.

Materials and Methods

Use of an economic threshold with *B. thuringiensis*

Spring 1989. Studies were conducted in 1989-90 at the Wes Watkins AREC, Lane, Oklahoma. Cabbage (cv. Express) was transplanted in single rows into raised beds set at 1 m spacing on 1 May 1989 using 30 cm interplant spacing. Each plot consisted of two beds, 10 m long, and separated by two unplanted beds. Treatments were replicated four times and arranged in a randomized block design. Trifluralin was broadcast before transplanting at the rate of 0.84 kg AI/ha for weed control. Plots were fertilized in accordance with soil test results and OSU Extension recommendations (Kahn et al. 1990). Cabbage was irrigated as needed using a surface level trickle irrigation system.

Five insecticide treatment regimes were tested: (1) weekly applications of permethrin at 0.12 kg AI/ha; (2) weekly applications of *B. thuringiensis* var. *kurstaki* at 0.55 kg/ha (Dipel 2X, Abbott Laboratories); (3) applications of permethrin (same rate as in 1) when counts of lepidopterous larvae exceeded the designated economic threshold; (4) *B. thuringiensis* applications based on the economic threshold; and (5) untreated. The economic threshold used in treatment 3 and 4, a modification of the level used by Cartwright et al. (1987), was 0.5 larva/plant before plant cupping and 0.3 larva/per plant after plants reached the cupping stage. All insecticide applications were made with a CO_2-powered backpack sprayer equipped with two (TX-10) nozzles per bed and calibrated to deliver 327 l/ha using 4.2 kg/cm^2 pressure.

Insect populations were monitored twice weekly in each plot by examining five whole plants per plot. Counts were made of all lepidopterous larvae and pupae, and eggs of cabbage loopers were recorded. Counts of aphids and thrips were also taken but were not considered in the threshold decision-making. Cabbage heads were harvested from each plot on 26 and 30 June, and 5, 11 and 18 July. Heads were weighed and examined externally for damage by lepidopterous larvae and thrips.

Fall 1989: Cabbage (cv. Express) was transplanted in single rows into raised beds set at 1 m spacing on 30 August 1989 using the same planting configuration as in the spring 1989 study. Treatments were replicated four times and arranged in a randomized block design. Sethoxydim was broadcast 10 days after transplanting at the rate of 0.21 kg AI/ha for weed control. Plots were fertilized in accordance with soil test results and OSU Extension recommendations (Kahn et al. 1990). Cabbage was irrigated as needed using a surface level trickle irrigation system.

Treatment regimes, experimental design, sampling procedures and crop damage evaluations were identical to those used in the test conducted in the spring of 1989. Insect populations were monitored twice weekly in each plot by examining five whole plants per plot as described in the spring 1989 study. Cabbage heads were harvested from each plot on 7, 11, 20, and 27 November, 1 and 4 December.

Spring 1990: The study design was identical to that used in the spring of 1989. Plants were transplanted into plots on 14 May 1990. Trifluralin was broadcast before transplanting at the rate of 0.84 kg AI/ha for weed control. Insect counts were taken as described above beginning on 21 May 1990 and ending on 16 July 1990. Harvests were performed on 17, 23 and 30 July and 6 August.

Statistical and economic analyses

Data were analysed with ANOVA (SAS Institute, 1985) and Duncan's (1955) multiple range tests were used for mean separations. Economic analyses were conducted to determine the effects of each insecticide treatment regime on net returns. Assumptions used in the analyses were based on budgets prepared by OSU Extension (Schatzer and Motes 1988). Based on the budgets for transplanted fresh-market cabbage, preharvest costs (PHC) (less insect control costs) were assumed at US$1877/ha and US$1857/ha for the spring and fall crops, respectively. Fixed costs (FC) for production were assumed at US$695/ha for the spring and US$650/ha for the fall crop. Harvest, packing and marketing costs were based on tonnage; US$103/t for packing and marketing (PC), $46/t for harvesting (HC).

Insecticide costs were based on a local retail supplier average, US$20/ha per application for permethrin and US$30/ha per application for *B. thuringiensis*. An additional US$7/ha was included in the total insecticide cost (TIC) for application costs, as per the current custom application costs. For the threshold-based applications, a scouting fee (SC) of $25/ha for twice weekly monitoring (18 visits) was added.

Gross returns were calculated by multiplying the marketable yield (MY) by the assumed price of $264/MT. This price represents an average wholesale price for the respective harvest periods obtained from the Dallas market. Net returns(NR) were calculated by the following formula:

$$NR = GR-[(HC*MY)+(PC*MY)+PHC+FC+TIC+SC^{1}]$$

([1]Included for threshold-based sprays only.)

Economic impact of DBM in the southwestern USA

Information provided for this summary of changes in cabbage pest control programs was obtained through phone surveys of growers, agricultural consultants and pesticide application

professionals. Estimated hectarage represented in the survey includes 30% of cabbage grown in the Texas Lower Rio Grande Valley, 75% of cabbage in the Texas High Plains, and 75% of cabbage grown in Oklahoma. Total estimated production per year is about 7000 ha.

Results and Discussion

Spring 1989

Populations of lepidopterous larvae were relatively high in untreated plots, beginning with the 28 May sample date. Nearly all larvae observed in this test were CL (92%), except for a few DBM larvae. In untreated plots, the mean number of larvae per plant (LPP) remained above the designated thresholds (0.5 LPP precupping, 0.3 LPP after cupping) throughout the season except on 22 June. The seasonal mean number of larvae was significantly greater in untreated plots (Table 1); the *B. thuringiensis* threshold plots had the next greatest numbers and plots treated weekly with permethrin had the least number of larvae, averaged over the season. No significant difference was observed among treatment regimes in seasonal mean numbers of aphids. However, thrips populations were significantly lower in plots treated weekly with permethrin. In this study and all subsequent ones, *Frankliniella fusca* L. comprised >95% of the thrips observed, based on weekly collections of specimens, and the remainder were identified as *Thrips tabaci* Lindeman.

Table 1. Seasonal mean numbers of cabbage pests and associated damage to cabbage, Lane, Oklahoma, USA, 1989-90[a].

Season	Spray regime	Mean No./5 plants			% Unmarketable heads by cause		
		Lep. larvae	Thrips	Aphids	Total	Lep. larvae	Thrips
Spring 1989	Weekly-Permethrin	0.9 c	27 b	6.3 a	8 b	0 b	4 a
	Weekly-*Bt*[b]	2.3 bc	149 a	7.0 a	19 b	2 b	7 a
	Threshold-*Bt*	2.7 b	136 a	5.9 a	6 b	3 b	3 a
	Threshold-Permethrin	1.7 bc	110 a	7.1 a	7 b	1 b	4 a
	Untreated	5.2 a	132 a	6.5 a	84 a	82 a	1 a
Fall 1989	Weekly-Permethrin	0.1 b	0 a	0.7 b	0 a	0 a	0 a
	Weekly-*Bt*	0.5 ab	0 a	57 ab	0 a	0 a	0 a
	Threshold-*Bt*	0.8 a	0 a	387 a	11 a	5 a	0 a
	Threshold-Permethrin	0.7 a	0.1 a	11 b	0 a	0 a	0 a
	Untreated	0.9 a	0 a	37 b	18 a	13 a	0 a
Spring 1990	Weekly Permethrin	0.3 c	13 b	0 a	5 a	0 c	0 a
	Weekly-*Bt*	1.2 b	26 a	0 a	10 a	7 b	0 a
	Threshold-*Bt*	1.5 b	30 a	0 a	16 a	9 b	0 a
	Threshold-Permethrin	0.9 bc	24 ab	0 a	8 a	4 bc	0 a
	Untreated	3.5 a	30 a	44 b	44 b	15 a	0 a

[a]Means within a column with the same letters are not significantly different among treatment regimes for each season, (P = 0.05, DNMRT). [b]*B. thuringiensis*.

The average weight per head was not significantly different among the spray regimes. However, the percentage of marketable heads was significantly lower in the untreated plots compared with all other treatment regimes (Table 2). This reduced marketability resulted in lower marketable yield calculated on a per hectare basis. Seven applications were required in each of the weekly treatment regimes. Two applications were needed in the threshold-permethrin plots and five sprays were needed in the threshold-*B. thuringiensis* plots. Insecticide costs were greatest with the weekly treatment of *B. thuringiensis* and were least in the plots treated with permethrin on a threshold basis. The threshold-based spray regimes resulted in higher net returns than the two weekly schedules and the *B. thuringiensis* plots had slightly greater net returns than

Table 2. Economic analysis of insect control strategies on fresh market cabbage, Lane, Oklahoma, USA, 1989-90.[a]

Season	Spray regime	Marketable heads (%)	Marketable yield (t/ha)	No. sprays	Insecticide cost (US$/ha)	Net return (US$/ha)	Break-even prices (US$/kg)
Spring 1989	Weekly-Permethrin	92.3	25.5	7	190	557	0.26
	Weekly-B.t.	81.2	25.9	7	259	89	0.28
	Threshold-B.t.	93.9	26.3	5	185	595	0.26
	Threshold-Permethrin	93.5	25.8	2	54	711	0.26
	Untreated	15.8	4.5	0	0	-2004	0.73
Fall 1989	Weekly-Permethrin	100	31.3	8	217	1155	0.25
	Weekly-B.t.	100	31.3	8	296	1076	0.25
	Threshold-B.t.	92.5	29.8	2	74	1085	0.25
	Threshold-Permethrin	100	31.3	2	54	1293	0.24
	Untreated	87	27.2	0	0	862	0.25
Spring 1990	Weekly Permethrin	95.4	6.0	9	245	-2071	0.63
	Weekly-B.t.	90.5	5.6	9	333	-2198	0.68
	Threshold-B.t.	83.9	5.2	3	111	-2052	0.68
	Threshold-Permethrin	91.9	5.5	2	54	-1961	0.64
	Untreated	55.9	3.5	0	0	-2135	0.90

[a]See text for explanation of computations.

the permethrin-treated plots. Break-even costs for all treated plots were nearly identical, ranging from US$0.26 to 0.28/kg, although the break-even price for untreated cabbage was substantially higher as a result of insect damage losses.

Fall 1989

Populations of lepidopterous larvae were lower than in the spring 1989 test. In untreated plots, moderate numbers of larvae were observed in mid September, early October and in mid October. As was observed in the fall 1989 test, most of the larvae observed in this test were cabbage loopers (91%), except for a few DBM larvae. In untreated plots, the mean number of LPP exceeded the designated thresholds (0.5 precupping, 0.3 LPP after cupping) only twice during the season. The seasonal mean number of larvae was significantly lower in the permethrin/weekly regime compared with untreated plots (Table 1). Aphid populations were greatest in the threshold/*B. thuringiensis* plots and lowest in the weekly/permethrin regime. Few thrips were observed in any of the plots.

The average weight per head of cabbage was not significantly different among the spray regimes, but heads were generally heavier than those harvested in the 1st spring test. However, the percentage of marketable heads was not significantly different among treatment regimes (Table 2); each treatment regime except the threshold/*B. thuringiensis* and untreated plots had 100% marketable heads.

Eight applications were required in each of the weekly treatment regimes. Only two applications were needed in the threshold-based plots (Table 2). Insecticide costs were greatest with the weekly treatment of *B. thuringiensis* and were least in the plots treated with permethrin on a threshold basis.

Net returns were greatest in the two weekly treatment regimes and the threshold/permethrin plot (Table 2). The threshold/*B. thuringiensis* regime and the untreated plots resulted in lower net returns than the other regimes. Break-even prices were similar for all treatment regimes.

Spring 1990

Populations of lepidopterous larvae were highest in untreated plots, averaging 3.5 larvae/5 plants and peaked on the 8 July sample date. More diversity in the species complex of larvae was observed; DBM larvae accounted for 28% of total larvae counted seasonally, while CL made up 59% and the remaining 13% was cabbage webworm. Thrips populations were significantly lower in the plots treated with permethrin on a weekly basis, but not different among all other treatments. The percentage of marketable heads was significantly lower in the untreated plots but was not different among any of the insecticide regimes. In general, yields were substantially lower in all treatment regimes as compared with the previous two seasons. The lower yield was the result of delayed planting because of early spring rainfall and reduced plant growth because of unusually heavy rains throughout the season. As a result of the lower yields, net return values were all negative and were lowest for untreated plots (Table 2). The plots treated with permethrin on a threshold basis provided the best net return and resulted in the lowest break-even price. Because yields were relatively poor, break-even prices were much higher than in previous seasons, ranging from US$0.63/kg in the threshold-permethrin plots to US$0.90 in the untreated plots.

In a similar study which attempted to integrate the use of damage-based thresholds with the use of different insecticide classes, Workman et al. (1980) found that spray programs based on visual damage thresholds reduced the numbers of insecticide applications but failed to produce high-quality cabbage. In nearly each case reported by Workman et al. (1980), the number of *B. thuringiensis* sprays required in threshold-based plots was higher than that of permethrin. Our results were similar to theirs; use of the economic threshold reduced the need for insecticides, regardless of type. However, unlike the results of Workman et al. (1980), the percentage of marketable heads was not reduced with threshold-based sprays. In addition, the number of sprays required in *B. thuringiensis*-treated plots were greater than in permethrin-treated plots. In each study, populations of larvae in *B. thuringiensis*-treated plots were consistently higher than those in the permethrin-treated plots. Although populations appeared to be higher in these plots, it did not result in significantly greater damage than was observed in permethrin plots. It is likely that moribund, nonfunctional larvae were included in the counts of *B. thuringiensis*-treated plots, but these larvae did not contribute much damage after exposure to *B. thuringiensis*. It appears that the economic threshold used in this study is effective regardless of the type of insecticide used.

In interpreting net return differences among treatment regimes, it is important to note that although some differences occurred, the calculations are based on plot yields and percent marketable heads in which the only significant difference observed was with the untreated plots. We observed that net returns were generally higher with the permethrin-threshold regime compared with others tested, reflective of reduced costs relative to other regimes. Therefore, under the conditions of our studies with relatively susceptible insect populations, carefully timed sprays of permethrin provided the most cost-effective approach to controlling lepidopterous pests of cabbage. However, with the onset of DBM resistance to insecticides in the southwestern United States, alternation of insecticide classes has become a necessity. If permethrin remains effective against these pests, it provides the best return on investment by growers.

Economic impact of DBM resistance in the Southwestern United States

A Review of Changes in Control Practices From 1986 to 1990. Prior to 1987 when resistant DBM became prevalent, the typical control program for most cabbage growers in the southwestern USA included weekly applications of pyrethroids (either permethrin or esfenvalerate), carbamate (methomyl) or sometimes an organophosphorus (methamidophos or mevinphos). In a typical control program in the region, the total number of applications ranged from 4 to 10 and usually included three or more pyrethroid applications. Before the onset of control problems, little use of *B. thuringiensis* products occurred, primarily because equal or greater efficacy could be achieved with other insecticide classes. Use of endosulfan was also

largely ignored, presumably because of marketing reasons; endosulfan was not actively promoted by agricultural chemical distributors as were some other products.

When control problems were initially reported in Texas, the first response of pest control advisors was to increase rates and frequency of applications of insecticides; secondarily, switching insecticide classes was attempted. However, as control difficulties became more common and the strategy of increasing rates of pyrethroid, carbamate and organophosphorus insecticides proved unsuccessful, other options were sought.

Current control programs in the Texas High Plains and the Texas Lower Rio Grande Valley reflect the significant change that has been required by the increased prevalence of insecticide-resistant DBM.

Case study: Texas High Plains-DBM resistance present

In the High Plains, the average number of insecticide applications increased from 10 per season to 15 with the increase in severity of DBM (Table 3). Total amounts of insecticide used increased from 4.3 kg/ha/season before DBM control problems were observed to 15.2 kg/ha in 1990. Similarly, insecticide costs rose from US$119/ha to US$254/ha, largely as a result of increased control costs associated with DBM. Changes were also noted in the classes of insecticides used for pest control on cabbage. A decrease in reliance on pyrethroids occurred and at the same time, an increase in the use of other classes occurred, especially increased use of methomyl and *B. thuringiensis*. Mixtures of insecticide classes within a single application have become more common.

Table 3. Insecticide use patterns on fresh market cabbage in the southwestern United States, in reference to DBM control problems.

	Average number of applications per season					
	Texas High Plains[a]		Oklahoma[b]		Rio Grande Valley (TX)[a, c]	
By Target Pest	1986	1990	1986	1990	1986	1990
Lepidopterans	7	12	4.5	5.5	5	11
Aphids	2	1	1	1	2	2
Other	2	3	1	1	2	2
By Insecticide Class						
Pyrethroid	7	5	4.5	3	4	4
Organophosphate	3	7	1	1	2	2
B. thuringiensis	0	6	0	4	1	8
Organochlorine	1	1	0	1	0	6
Carbamate	1	11	1	2	4	4
Total Applications:	10	15	6.5	7.0	8	13
Total Amount of Insecticide (kg/ha/season)	4.3	15.2	2.5	7.4	5.7	18.1
Total Cost: (US$/ha/season)	262	578	54	97	370	518

[a]Control failures with DBM noted in 1987. [b]No control problems noted to date. [c]May involve multiple insecticides per application.

Case study: Texas Lower Rio Grande Valley-DBM resistance

In the Lower Rio Grande Valley of Texas, traditionally the largest cabbage production area of the southwestern United States, major changes have occurred as a result of the increase in pest status by DBM. Until 1986, 6-8 insecticide applications were typical, with two early applications for aphid control followed by 4-5 applications for lepidopteran control (Table 3). Beet armyworm (BAW) (*Spodoptera exigua* Hübner) and CL constituted the major part of the lepidopterous pest complex; DBM larvae were not present in sufficient numbers to warrant insecticide applications. The relatively few DBM present were easily controlled by methomyl

or pyrethroids. *Bacillus thuringiensis* products were rarely used, primarily because of their lack of efficacy on BAW and thus more cost-effective control was obtained without them.

In 1986, a significant shift in cabbage pest control in the Rio Grande Valley occurred with the onset of DBM-related control difficulties (Table 3). By 1986, eight applications were needed, including several mixtures of insecticides. By the 1989-90 season, an average of 12-15 total insecticide applications were needed to manage cabbage pests, with as many as 20 sometimes needed. Concurrent with the increased number of applications and total amount used, considerable change in the types of insecticide was required. Use of *B. thuringiensis* products was a mainstay in most insecticide applications, and use of endosulfan greatly increased. Pyrethroid use declined precipitously; they were only used for CL control as needed. Methomyl and mevinphos remained partially effective against DBM and were used as rotational materials.

During the period 1986-88, a dramatic shift in the lepidopteran pest complex occurred. DBM activity steadily increased, replacing BAW as a primary pest which became a secondary pest. By the 1988-89 season, BAW was greatly reduced in pest status, apparently controlled by the increased pesticide load required for DBM control. DBM is now the key pest species of the lepidopterous complex.

In addition to increased insecticide inputs on cabbage, control problems with DBM have generally resulted in decreased yield and quality of cabbage grown, with an estimated average yield loss of 30-40% in the two resistant areas. This has led to a 40% projected decrease planting of cabbage in the 1990-91 season.

Case Study: Oklahoma-No DBM resistance

In a national bioassay of DBM populations in 1988 conducted by A. Shelton and J. Wyman, DBM collected from Oklahoma showed RR values less than 0.5. However, growers and crop consultants in Oklahoma are aware of the control difficulties experienced by their counterparts in Texas and have made adjustments in the types of insecticides used on cabbage in a preemptive effort to avoid DBM control failures. Because prevailing weather conditions threaten to spread insecticide-resistant DBM populations northward to Oklahoma, growers have reduced their reliance on pyrethroid insecticides and have incorporated the use of *B. thuringiensis* products into most of their applications on cabbage (Table 3). This shift toward using a diversity of insecticide classes on cabbage should help avoid future control problems with DBM, assuming registrations for the use of these products can be maintained.

In summary, the DBM control failures that were first noticed in 1987 have led to increased amounts of insecticide use, decreased yield and profitability, and appear to cause significant concern for the cabbage industry in two production areas of southwestern USA. The effect of DBM control problems on cabbage production in the southwestern United States has been profound. In addition to increasing the costs of control, the marketability and yields of cabbage have suffered in the areas where control difficulties were the greatest. Growers and pest control professionals are now looking for alternative methods of control. Until improved technology is available, the amount of cabbage grown in the region is likely to continue to decline. For most seasons, cabbage is a marginally profitable crop and as these control difficulties continue, growers will likely shift away from cabbage production as a result of the relatively high risk compared with anticipated profit.

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INSECTICIDE RESISTANCE

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Esterase Isozyme of Diamondback Moth

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Abstract

Electrophoresis is currently the best technique for quantifying inherited variation which has contributed to our knowledge of population biology in revealing population structure. Considerable variation in isozymes of diamondback moth, *Plutella xylostella* (L.), was detected. Esterase zymograms were prepared from 3rd and 4th instar larvae, pupae and adults. There were two groups of esterase bands, which appeared to be independent. The faster band was simple to analyze genetically and involved at least six alleles. In a preliminary study, esterase zymograms of four populations, collected in Shimane and Osaka prefectures of Japan, were compared using these alleles. Gene frequency was different between the four populations, suggesting that esterase isozymes could be useful in analyzing the population biology of diamondback moth.

Introduction

Diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), is a serous insect pest of crucifers. DBM has developed high levels of resistance to various insecticides (Hama 1987). Tanaka and Kimura (1990) reported a decrease in susceptibility to *Bacillus thuringiensis* Berliner insecticide (Toarow CT wettable powder). Hama et al. (1990) found that the *B. thuringiensis* resistance in DBM is genetic. Ecology, occurrence and control of DBM in Japan has been widely studied (Sakai 1988). The migration of DBM is known in Europe (Williams 1958), however, there is no evidence of DBM migration in Japan. Honda and Miyahara (1987) suggested that DBM may migrate from southern areas to northern Japan. It is not clear, however, that DBM do, in fact, migrate. Isozyme analysis could be used to identify local populations and monitor any large-scale migration.

Isozyme analysis has contributed not only to discrimination of insect species but also analysis of population biology in many insect species (Loxdale and Hollander 1989). In this paper, isozymes of DBM were studied as a means of analyzing population structure and insecticide resistance.

Materials and Methods

Insect source

The origins of four populations of DBM used in this study are shown in Table 1. These populations were reared on Japanese radish seeds cultured in plastic vessels in a chamber at $23 \pm 1°C$ and 16L:8D. Adult moths were stored at -30°C before being homogenized.

Table 1. Origins of DBM.

Population	Locality	Date collected	Resistance type[a]
OS	Osaka	June 1990	BT, C
MA	Masuda	23 March 1990	O, P
YO	Yokota	6 September 1990	O, P, C
MT	Matsue	23 March 1990	O, P

[a]BT, *B. thuringiensis*; O, Organophosphorus; P, Pyrethroid; C, Cartap.

Electrophoresis

Adult moths were homogenized individually in 20-40 μl mixture of 20% sucrose solution and 0.25% Trixton X-100, and 10-20 μl homogenates were dispensed into pockets in the gel. Electrophoresis was carried out at constant current (30-40 m A) for about 1 hour on 7.5-9% polyacrylamide vertical slab gel, using Tris glycine (pH 8.6) as running buffer. Gels were then stained for 10 min at 36°C for esterase with Fast Blue BB or RR salt in 0.1 M phosphate buffer (pH 7.0) containing 1-naphthyl acetate (dissolved in acetone).

Results and Discussion

Esterase zymograms of DBM were detected in larva, pupa and adult stages. Esterase isozyme patterns of DBM are shown in Fig. 1. There were two distinguishable banding groups (Est-1, Est-2) on the esterase zymogram. These band groups were independent of each other. A slower band group (Est-2) was clear and had many bands. A faster band group (Est-1) was slightly dim and each band was represented by a double band (doublet). In the Osaka population, 9 of 11 adults had the same band in Est-2. However, the Matsue population had different band patterns in Est-2. There was wide variation on Est-2 of the four populations examined in this study. The Osaka population was heavily selected with *B. thuringiensis* application. Therefore, there might be a simple band pattern on Est-2 loci of the Osaka population. A band pattern of Est-1 loci was simple and useful to determine heredity of DBM. It was clear that there could be at least six bands in Est-1 loci.

In crossing tests, theoretical genotype appeared in the F_1 generation. Esterase zymograms of parent and F_1 are shown in Fig. 2. Parents of genotype BB and AC produced AB and BC genotypes in F_1. Therefore, bands of Est-1 must be alleles on Est-1 loci. Genotype frequency is shown in Table 2. There are six alleles and 21 genotypes in Est-1 loci. Eighteen genotypes were detected in four populations. Osaka and Matsue populations had 12 genotypes, and Masuda and Yokota populations had eight genotypes, respectively. Gene frequency of allele is shown in Table 3. In Osaka and Yokota populations, there was high frequency of B allele. Matsue and Masuda populations had a high frequency of C and A alleles, respectively.

These alleles could be available as genetic markers of population charactertics and these markers could be applied to population analysis of DBM with regard to migration and distribution.

Est-2 band of the Osaka population showed homozygote alleles. Hama (1990) reported that *B. thruingiensis* resistance of DBM is incompletely recessive. Therefore, this resistance will appear on homozygote genes. Homozygotes of Est-2 may be coincident with the *B. thuringiensis* resistance gene. However, there is no evidence of coincident homozygote genes. The Osaka population was selected by *B. thuringiensis* (Tanaka 1990), suggesting little genetic variation. If Est-2 loci coincides with *B. thuringiensis* resistance, it will be possible to monitor the change in resistance gene frequently resulting from insecticide selection.

Isozyme analysis was applied to identification of species and biotype, and population genetic studies on many insects such as aphids, egg parasite (*Trichogramma* sp.) and so on (Loxdale and Hollander 1989). This has not been atttempted on DBM. Honda and Miyahara (1987) reported that DBM cannot hibernate in Tohoku district, and they suggested that DBM might migrate

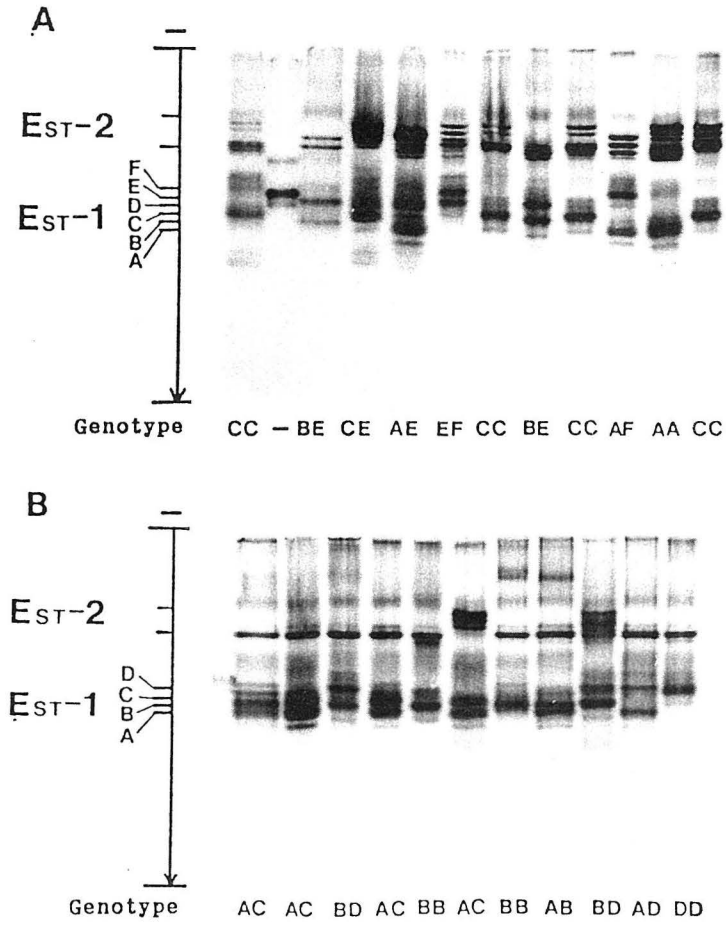

Fig. 1.
Esterase zymograms of two DBM strains: A, Matsue; B, Osaka.

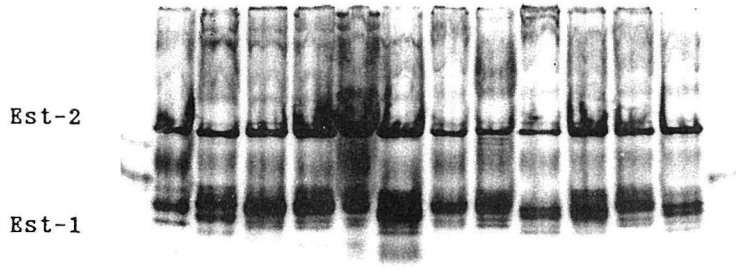

Fig. 2.
Esterase zymogram of parent (P) and F_1 of DBM (Osaka).

Genotype BB AC BC BC BC AB BC BC AB AB BC AB

P F1

Table 2. Genotype frequency in Est-1 loci of DBM.

Genotype	Genotype frequencies of each strain			
	OS	MA	MT	YO
AA	1	5	1	0
AB	1	0	0	0
AC	6	4	0	2
AD	4	3	0	1
AE	0	0	1	0
AF	0	0	1	0
BB	15	0	2	4
BC	4	3	0	4
BD	14	1	0	6
BE	3	0	2	1
BF	0	0	1	0
CC	3	1	7	0
CD	2	4	0	0
CE	2	1	3	3
CF	0	0	0	0
DD	1	0	1	0
DE	0	0	1	0
DF	0	0	1	1
EE	0	0	0	0
EF	0	0	1	0
FF	0	0	0	0

OS, Osaka strain; MA, Masuda strain; MT, Matsue strain; YO, Yokota strain.

Table 3. Gene frequencies in Est-1 loci of DBM.

Strain	No. of individuals	Gene frequency of allele					
		A	B	C	D	E	F
OS	56	0.116	0.464	0.178	0.196	0.045	0
YO	22	0.068	0.432	0.205	0.182	0.091	0.023
MT	22	0.091	0.159	0.386	0.091	0.182	0.091
MA	22	0.386	0.091	0.318	0.182	0.023	0

from southern to northern Japan. However, there is no evidence that populations do migrate. In future, isozyme analysis might be used with DBM to estimate the genetic differences between different populations, and to study the levels of genetic variation and the distribution of allele frequencies in natural populations.

Conclusions

Esterase isozymes were studied on larva, pupa and adult DBM. There were two band groups, a faster band (Est-1) and a slower band (Est-2). Est-1 was used to analyze genetic variations. There were at least six alleles on Est-1 loci. These alleles were useful in analyzing the different gene frequencies in four populations. Est-1 bands could be useful as markers to show characteristics of regional populations. The Osaka population, which is resistant to *B. thuringiensis* might coincide with Est-2 loci.

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Esterase Zymograms as an Assay for Detection of Resistant Populations of Diamondback Moth

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Abstract

Five strains of Sheh-Tzu diamondback moth, *Plutella xylostella* (L.), showing different levels of malathion resistance, were successfully obtained by single-female mating method within 4 years and about 65 generations, although no insecticide had ever been applied to this population. The difference in LD_{50} of malathion between the most resistant strain and the susceptible strain is 22-fold, and the difference in adult emergence rate of the treated larvae between two strains is 900-fold. Analysis of the frequency of esterase isozymes in larval zymograms showed that the resistant larvae possessed an extra high frequency of Est 8/Est 9p. Six intra- and interbreeding experiments of several strains by single-female mating have been carried out to determine how Est 8 and other isozymes were involved in the resistance of the larvae. Est 8 was found to be significantly negatively correlated with aberrational growth of the treated larvae of susceptible strains. Twenty-two wild populations of the diamondback moth around Taiwan were tested for correlation between isozyme frequency and malathion resistance. Increased frequency and the titer of the Est 9p in the larval zymograms were significantly correlated with the increased resistance of the larvae to malathion. The LD_{50}s of each of 17 wild populations of diamondback moth to mevinphos, fenvalerate, carbofuran and permethrin were also plotted against the isozyme frequency in larval zymograms of each of these 17 populations. Results showed that increased frequency of Est 9p in the larval zymograms was significantly correlated with the increased resistance of these populations to mevinphos and fenvalerate, but were not correlated with permethrin and carbofuran. Amplification of Est 9 was found in the larvae descended from the second generation of intrabred broods from many strains. Amplification of either Est 4 or Est 9 would elevate or deprive other esterase isozymes, including Est 8 and Est 9p. This phenomenon suggested that insecticide application to control the pest in the field would eventually support a situation for Est 8/Est 9p elevation in the diamondback moth larvae which would result in the emergence of resistant populations in the field.

Introduction

Insect esterases perform both physiological and defensive functions and are found in both soluble and membrane-bound forms. Among insect species, the carboxylesterase of green peach aphid (Devonshire and Sawicki 1979), *Culex* mosquito (Georghiou and Pasteur 1980), housefly (Oppenoorth 1965), leaf hoppers (Ozaki and Kassai 1970) and cutworm (Bull and Whiten 1972) have been studied extensively because of their involvement in resistance to insecticides. Recent reports indicate that amplification of esterase genes are responsible for insecticide resistance in California *Culex* mosquito (Mouches et al. 1986) and in green peach aphid (Field et al. 1988).

Enhanced esterase activities were also found in the malathion-resistant diamondback moth (DBM) *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) (Doichuanngam and Thornhill 1989; Maa et al. 1983). Maa et al. (1990) reported that 17 esterase isozymes were detected

in the soluble fraction of the larval homogenate of DBM. They revealed that higher hydrolytic activities of membranous esterases were bestowed upon resistant larvae of Sheh-Tzu (ST) populations. The total soluble esterase activities of ST larvae to 1-naphthyl acetate is somehow the same as that of Geou-Fang (GF) larvae, a susceptible population.

Two soluble esterase isozymes, designated Est 8 and Est 9p, were suggested to be crucial to resistance of the ST larvae. The frequency of Est 8 in ST larvae was significantly higher than that in GF larvae. Esterase isozymes of the ST population were more tolerant to various esterase inhibitors than those of the GF population. On the other hand, the treated GF larvae which were liable to malathion-induced neurosecretory toxicity resulted in malformed larvae that resemble larva-pupal intermediates. The aberration was presumed immediately correlated with low frequency of Est 8 and low paraoxon-tolerance of Est 9p in the GF larvae (Maa C.J.W., unpublished).

Isozymes were concerned with identification of allelic frequencies at the level of hybrids of substrains, strains and natural populations. For those involved in resistant discrimination at much finer levels as hybrids of substrains may be of crucial importance. What sort of allozyme or isozyme differences exist between these hybrids, substrains, and strains, and can these differences be exploited for discrimination of insecticide resistance? Such questions are particularly amenable to isozyme analysis since isozyme markers have already been used to distinguish races of the European corn borer which respond differentially to sex pheromone (Garde et al. 1978), the strains of the green peach aphids which resist differentially to various synthetic pesticides (Devonshire and Moores 1982), and the *Culex pipens* complex, from various geographic areas, which resist organophosphorus insecticides (Fournier et al. 1987).

In this study malathion was not used for selection but for monitoring the resistance of the identified strains. Strains were obtained by single-female pairing method rather than group mating unless otherwise mentioned. Five strains and four substrains from the ST population were obtained. Selection was based on two factors: (1) toxicity effect of malathion to the larvae, and (2) frequency and intensity of Est 8/Est 9p, as marker proteins, in the larval zymograms of ST broods.

Intrabreeding of different DBM strains has been carried out. We now report that resistant organisms are derived by intrabreeding of either resistant strains or susceptible strains, through the selection for high titer of specific esterase isozyme. We found that the resistant organisms differed from the susceptible organisms in possessing enhanced Est 9p as well as Est 8.

Reciprocal crosses were also made between the parent stocks of different strains, substrains, or broods of F_1 hybrid progenies. Toxicity tests and zymogram studies were done. The 84-hour old 4th instar larvae were used throughout the study. Twenty-two natural populations of DBM in Taiwan were collected and the LD_{50} of these populations to malathion was investigated. The frequency of Est 9p alone of the larvae was also tested as to whether this enzyme could be used as an indicator protein to monitor the resistance of DBM larvae to synthetic pyrethyroids, carbamate or organophosphorus insecticides.

Materials and Methods

Insects

DBM collected from vegetable farms in Taiwan were reared under constant temperature of $24 \pm 1°C$ and 14L:10D conditions. The larvae were fed with rape seedlings (Koshihara and Yamada 1981). The last instar 84-hour-old larvae were used for susceptibility tests and for esterase zymogram study according to Maa et al. (1990).

Isolation and selection of resistant and susceptible strains

The ST strain developed resistance due to heavy insecticide application for DBM control during the last decade. The larvae were resistant to various insecticides including fenvalerate,

mevinphos, carbofuran, permethrin (Cheng 1981) and malathion (Maa et al. 1986). The ST larvae were, however, rather heterogeneous in malathion resistance (Maa et al. 1988). Isolation and selection of malathion-resistant or malathion-susceptible strains from ST populations of DBM were initiated by single-female pairing. The selection was based on two parameters: (1) the susceptibility of the larvae to malathion, and (2) intensity and frequency of the soluble esterase isozymes in PAGE zymograms of the larval homogenate.

Those larvae that descended from each single-paired mate were treated topically with 33 μg malathion per larva. Thirty larvae were used for each assay. After the treatment the larvae were confined within a Petri dish (6.0 cm diam and 1.5 cm high), and kept in the above-mentioned conditions for observation for 2 days. Mortality of the larvae due to either acute effects or induced effects of the insecticide was recorded. Any brood of sister-larvae that was neither significantly susceptible nor resistant to malathion was discarded. Treated larvae were killed in order not to gain resistant individuals through the application pressure. Twenty sister-larvae were needed for zymogram study. It is necessary to keep at least 24 additional larvae for propagation. All the larvae used for the susceptibility test, zymogram study and propagation should be sister-larvae of a brood descended from one single-paired mate. Those DBM larvae which were highly susceptible or resistant were allowed to develop into virgin moths, mated in groups and had progenies for further selection. Fourteen broods of ST strain were utilized for further selection.

More than 300 individual larvae, descended from the same brood, were used for the LD_{50} assay. Batches of 30 larvae were treated topically with malathion in two series of concentrations: 0.5, 1.6, 4.0, 8.3, 16.5, 33.0 μg/larva for the susceptible strains and 16.5, 33.0, 88.0, 132.0, and 176 μug/larva for the resistant strains. In this case the larvae were checked every 12 hours until the insect emerged into adults. Dosage-mortality curves of LD_{50} were calculated using probit analysis method proposed by Finney (1971). The lethal dosages to adult emergence were also calculated by using Abbot's formula. Larvae which were significantly resistant to or susceptible to malathion were therefore maintained and reared as newly selected strains. Identification of strain was dependent upon the zymogram pattern of the larval homogenate.

Comparison of esterase pattern between progenies

Three strains were either interbred or intrabred for one, two or several generations depending on the purpose. The breeding was also done by means of single-female pairing. The resistant larvae descended from either broods were treated with 66 μg malathion for susceptibility testing. The susceptible broods were treated with 6 μg malathion. Mortality rate of the larva was calculated. The esterase zymograms of the larvae were investigated. The frequency and the intensity of every esterase isozyme were carefully evaluated for elevated or amplified hydrolytic activities to 1-naphthylacetate (NA) in the zymograms.

Frequency of Est 9p in different DBM populations

Twenty-two DBM populations were collected throughout Taiwan during the spring season. Progenies of the 1st generation decended from each population were used for susceptibility testing and zymogram study, to determine whether Est 9p or any other esterase isozyme alone could be used as a marker protein to monitor the resistance of DBM to insecticides other than malathion.

Chemicals

All chemicals and reagents were of analytical grade or reagent grade. Diazoblue, lauryl sulfate, fast blue RR, eserine, parahydroxyl mercuribenzoate (PHMB) and other chemicals were purchased from Sigma Chemical Company USA. All chemical reagents for electrophoresis were purchased from Bio-Rad Laboratory, USA. Paraoxon (O, O-diethyl-o-p-nitrophenyl-phosphate), malathion (O-dimethyl-S-(1, 2-dicarboethoxyl) dicarboethoxyethyl phosphorodithioate) were purchased from Chemical Service Company, USA.

Zymogram Study

Larvae of the selected strains, substrains, the hybrids and the field populations were used. Samples for zymograms were prepared and run into PAGE according to procedures developed by Davis (1964) and Maa et al. (1990). Esterase isozymes were prepared and stained with 1-NA according to Ogita and Kassai (1965). Paraoxon and eserine were used to characterize the esterases in the gel. Parmacia Electrophoresis apparatus GE-24 with 2-mm thick gel was used for isozyme separation throughout the experiment.

Data Analysis

Correlation between mortality rate of the treated larvae and the frequency of each esterase isozyme of the larvae were assayed by linear regression. The correlation between isozyme frequency and the rate of aberrational immature and the rate of adult emergence respectively were also analyzed. Correlation between the LD_{50} to malathion of the larvae from field populations and the frequency of Est 9, in the presence of paraoxon, was analyzed accordingly. Lethal concentrations of mevinphos, carbofuron, fenvalerate and permethrin to the larvae of the DBM populations from Cheng (1981) were transformed into log value for linear regression assay.

Results and Discussion

Intoxification, zymogram and resistance

It is difficult to differentiate the acute toxicity of malathion, observed within 24 hours after treatment, from the induced neurosecretory toxicity observed throughout the immature to adult stages of DBM. Malfunction of neurosecretory systems were hypothesized to be involved during the intoxification of the caterpillars, especially when organophosphorus insecticides were used (Maddrell and Rey 1972).

Nevertheless, death of the treated DBM larvae due to acute toxicity was encountered when the immature ones became melanized and dehydrated into miniature carcasses. This was found when high doses (above 16 μg/larva) of malathion were applied to the larvae of both resistant and suceptible strains. Similar results were obtained in susceptible individuals when low doses (<1 μg/larva) of malathion were used. Larvae affected by the induced toxicity usually grew into aberrative forms. All the aberrant immatures would die eventually. On the other hand, when low doses of malathion were used on the resistant larvae, acceleration of pupation occurred in some individuals. The miniature pupa would, however, emerge successfully into adult stage.

The evidence suggested that insensitivity of the target organ, possibly the neurosecretory or growth-related hormone system, of the insect to malathion or to the derivatives of malathion, was possibly one of the major factors in the resistance mechanism of DBM. Another factor concerned with malathion-resistance of DBM was possibly elevated esterase activity of the larvae. Other resistance mechanisms were certainly involved, but were not explored in this study.

Esterase 8 was the most important isozyme associated with aberrational growth of larva. We found that of the treated larvae, those that died within 3 hours possessed low frequency (lower than 30%) of Est 8, and those that survived for 4 hours possessed high frequency of the same isozyme (about 80%). Coincidentally, GF larvae also possessed low frequency of Est 8 and became aberrant when the larva was exposed to malathion (Maa et al. 1990). In addition, Est 8 gradually became indistinguishable from the zymogram of body homogenate as the larva emerged into a prepupa. Est 8 was, therefore, presumed to be associated with target-insensitivity of the larvae to malathion or its derivatives.

The band coded for Est 9 actually contained at least two isozymes with different chromatographic properties. These isozymes were superimposed in the same area on the gel.

In the absence of genetic data, it is difficult to attribute esterases to given loci on the basis of differences in electrophoretic mobility alone. However, because of obvious differences in staining intensity of these isozymes, and in sensitivity of these isozymes to paraoxon, these two isozymes were designated as Est 9p and Est 9np, respectively. The former one was responsible for tolerance to paraoxon and stained lightly with NA and the latter was sensitive to paraoxon and stained intensively with the dye. Partially purified Est 9p was capable of hydrolyzing malaoxon as well as malathion (Maa unpublished data). We expected that Est 9p might play a dual role in malathion and malaoxon, degradation in DBM or as a protein indicator for malathion resistance at a moderate level.

Strain identification and esterase isozymes

Data in Table 1 show that based on LD_{50}, the larva of ST10 strain was 22 times more resistant than ST12 strain. The ratio between ST26 or ST34 larvae and ST12 was about 14. Comparison of dosage-mortality parameters (Table 1) or isozyme pattern of soluble fractions (Fig. 1) of these strains show that the ST34 larvae were like the ST26 larvae in many respects, including LD_{50}s, slopes of dosage-mortality curves, and the properties of the Est 7, 8 and 9p. In addition, the Est 8/9p pattern and the mortality rate of the hybrids of ST12 × ST26 were also the same as that of the ST12 × ST 34 (data not shown). These data indicated that analog mechanisms of resistance were likely bestowed upon the larvae of ST26 and ST34 strains.

Different isozyme patterns were evidently bestowed upon the larvae of different strains. Zymogram studies revealed that all resistant larvae possessed Est 8 and Est 9p, but only 27% of ST12 larvae had Est 8. Est 8 and 9 in ST12 and ST15 larvae, however, were susceptible to paraoxon. Est 7 of ST15 larvae was heavily stained with 1-NA. This esterase band was, however, dim and barely detectable in larval zymograms of other strains.

The ratio of the rate of adult emergence between ST10 and ST12 was 920, and the ratio between ST26 (or ST34) and ST12 was 305; a threefold difference. Variation on resistance between ST10 and ST26 larvae was not yet clear. Nevertheless, Est 9p of ST10 larva was more tolerant to paraoxon than that of ST26 larva, and ST10 larvae had both Est 8/Est 9p intensively stained in the zymogram. A higher frequency of Est 8/Est 9p in zymograms of ST 10 larvae was somehow associated with malathion resistance of ST10 strain. Est 8/Est 9p were likely usable as indicator proteins to monitor the resistance of DBM. Additional studies on isozymes as indicator proteins have been carried out.

Table 1. Strain-dependent variation on distribution of esterase isozyme and susceptibility of DBM larvae to malathion.

Strain	LD_{50} (μg/larvae) mean + SD		Slope at		Frequency of		
	24 h	emergence	24 h	emergence	Est 7	Est 8	Est 9 (9p)
ST12	6.2+2.3	0.06+0.03	0.79	1.88	.78 (dim)	.27	1.00(0)
ST15	4.7+1.8	0.17+0.15	0.69	2.23	1.00	1.00	1.00(0)
ST 26	88.5+9.3	18.30+7.60	1.95	4.26	.80 (dim)	.85	.93(+) (dim)
ST 34	82.4+11.6	16.98+6.40	1.92	4.23	.75 (dim)	1.00	1.00(+) (dim)
ST10	136.1+23.7	55.23+16.34	2.13	4.74	.76 (dim)	.81	1.00(+)

The larvae of ST12 and ST15 were treated with 5 doses ranging from 0.1 μg to 10.0 μg/larva. Those of other strains were treated with doses ranging from 16.5 μg to 176 μg/larva. Triplicate assays were done. (9p), (0); no Est 9p being detected in presence of paraoxon. (9p), (+); with Est 9p being detected in presence of paraoxon.

Correlation between isozymes and resistance

Three toxicological measurements listed in Table 3 were used to justify whether esterase isozymes found in the zymograms would correlate with these measurements. Larvae that died within 48 hours with or without symptoms of acute effect of the treatment were considered as poisoned by malathion. Those with aberrational symptoms broken off 48 hours after the treatment accounted for the effect of OP-induced neurosecretory toxicity. And those that pupated but were unable to emerge into adults were considered to have died of unknown chronic effects of malathion.

Table 2 shows that correlation between frequency of Est 8 and malathion resistance of ST12SS progenies was statistically significant with a positive fitness of 99%. Meanwhile, Est 9p frequency in the larval zymogram was also significantly correlated with the resistance. This suggested that Est 8 and Est 9p of ST12RR were expressed concomitantly by an unknown regulation mechanism(s) of the insect. Highly resistant individuals were recognized as those with intensively stained bands of Est 8/9p in the zymograms. Those larvae with Est 8 but without Est 9p in the zymogram were considered to be ones with lower resistance to malathion. Those that were lacking Est 8 were recognized as susceptible individuals.

The linear regression analysis assay shown in Table 2 was used as an example to illustrate how the correlation between frequency of Est 8/Est 9p and resistance of the larva was reciprocally interactive, and how the fitness of the correlation was to be justified.

Table 2. Correlation of Est 9p or Est 8 frequency of the ST12 larvae and the rate of adult emergence.

ST brood		Frequency of Est 8	Frequency of Est 9p	Rate of adult emergence
ST12RR				
	no. 8	.889	.667	.500
	no.15	.556	.500	.429
ST12RR f × ST12SS m				
	no. 1	1.000	.889	.846
	no. 4	.333	.000	.000
	no. 5	.000	.000	.000
	no. 6	.444	.444	.381
	no. 14	1.000	1.000	.667
	no. 15	0.556	.556	.724
ST12SS f × ST12RRm	no. 4	1.000	1.000	.833
	no. 13	1.000	1.000	.400

f; virgin female, m; virgin male, no; coded number of the mating pair. For Est 8 and emergence rate; $Y = .03 + .708X$, $r = .857$, $P < .01$. For Est 9p, $Y = 4.247 + .673X$, $r = .885$, $P < .01$, n;10, df; 8. Susceptibility assay; 66 μg/larva, 16 larvae for each brood. Zymogram assay; 9 larvae for each brood.

The same analytical method was applied for the bred progenies of other strains. Results of independent experiments on eight different strains or substrains are given in Table 3.

Results of the crosses between different ST strains were interesting in two respects. First, Est 8, as expected, was positively correlated with malathion resistance in all cases studied except ST12S, a substrain selected for absence of Est 8. Second, Est 4 was presumed to be negatively correlated with malathion resistance of the organisms derived from ST12 strain. The results of assays 3, 4 and 5 revealed that Est 4 was negatively correlated with resistance of the larvae in different levels from $<P.05$ for ST12R, $<P.1$ for ST12s. Correlation between Est 4 frequency and malathion-resistance was found insignificant in interbred progenies of ST12

Table 3. Linear regression correlation between aberration rate, mortality rate, or adult emergence rate of malathion treated larvae of ST strains and the frequency of esterase isozymes of the ST larvae.

Strain and the assay parameters	Coded isozyme	Aberration (%)	Mortality (%)	Adult emergence (%)
1st assay				
ST12, ST26, ST34	Est 3	--	no	no
ST12X, ST26	Est 4	--	no	no
ST12XST34				
n; 7, df; 5	Est 7	--	no	no
6 μg/larva	Est 8	--	-(<P.01)	+(<P.01)
2nd assay				
ST12, ST10,	Est 3	no	no	no
ST10XST12,				
ST10-1,	Est 4	no	no	no
ST10-2A4				
n; 14,	Est 7	no	+(P.05)	no
df; 12				
66 μg/larva	Est 8	-(<P.01)	-(<P.01)	+(<P.02)
3rd assay				
ST12R	Est 3	no	no	no
n; 9	Est 4	no	+(<P.0.1)	-(<P.05)
df; 6				
66 μg/larva	Est 7	no	no	no
	Est 8	-(<P0.1)	-(<P.01)	+(<P.02)
4th assay				
ST12S	Est 3	no	no	no
n; 8	Est 4	no	no+*	no-*
df; 6				
6μg/larva	Est 7	no	no	no
	Est 8	no	no	no
5th assay				
ST12S	Est 3	no	no	no
n; 9	Est 4	no+*	no+*	-(<P0.1)
df; 7				
66 μg/larva	Est 7	no	no	no
	Est 8	no	no	+(<P0.1)
6th assay				
ST12RR	Est 3	-(<P.01)	-(<P.01)	+(<P.05)
ST12SS				
ST12RR m ×	Est 4	no	no	no
ST12SS f	Est 7	no	no	no
n; 8, df; 6				
66 μg/larva	Est 8	-(<P.1)	-(<P.05)	+(<P.05)

+; positive correlation, -; negative correlation, --; not detected, no; not significant correlation, no-*; with highest value in negative correlation but not significant, no+*; with highest value in positive correlation but not significant, n; number of assays with 15 larvae used for each assay, df; degree of freedom, ST12 × ST 10; mating in single female pairing between ST12 and ST10 strains. The susceptibility bioassay was carried out under 20°C.

× ST26 or of ST12 × ST10 (see assays 1 and 2, Table 3). These results suggest that Est 4 of larvae susceptible to malathion was distinguishable only when homozygous individuals of susceptible populations were intrabred. In addition, elevated or amplified Est 4 was mostly found in zymograms of the intrabred progenies as well. It proved that Est 4 could only be used as a recessive indicator protein for malathion-susceptible DBM.

Results of assay 4 also reveal that larvae of ST12S were highly susceptible to 6 μg of malathion. Most of the treated larvae became larva-pupal intermediates and less than 25% of the treated immatures were able to emerge into adults. In these cases Est 8/Est 9p were not detected simultaneously in the zymograms of their sister-larvae. On the other hand, none of the aberrant immature was found in ST10 or other resistant larvae treated with 6 μg of malathion. Still, resistant larvae of prewandering phase would be, however, affected by malathion. These larvae would either enhance their development, and consequently emerge into miniature pupae, or would delay their maturation when a lower dose of malathion was applied to them. The acceleration in development of the larvae ended with pupae losing 20% of the body weight, compared with those of normal ones. Details of the effect of malathion on DBM larvae in the sense of disruption of the neurosecretory and endocrine systems were worthy of further investigation.

Another interesting aspect about the correlation was Est 3 and Est 7. Est 3 was found to be positively correlated with resistance in the broods of the hybrids of ST12RR and ST12SS only. Est 7 was stained intensively only in zymograms of the hybrid-progenies of ST 10 and ST 12.

Interbreeding of ST10 with ST12 in other cases also resulted in decreased resistance, and with Est 8 off from the corresponding site in the larval zymograms. Cheng et al. (1990) did report that the resistant DBM would gradually drop their resistance to pesticides if these resistant individuals were interbred with susceptible mates.

These results shed some light on speculation about resistance-dominated gene(s) being recessive. In other words, strengthening of DBM in pesticide resistance could be accomplished either by intrabreeding of resistant stock (e.g. ST 10 for one generation) or by continuously selecting a wild population of DBM with the same kind of insecticide for several generations until the resistance is achieved. Weakening of a resistant population can scarcely be done by just releasing the population from selection pressure, either biological or biochemical.

Thus, correlation between a specific isozyme and malathion resistance of DBM larvae could be illustrated through a comprehensive genetic study on strain selection and isolation, and intrabreeding and interbreeding of the selected strains. Study of esterase isozymes of DBM thus provided a simplified and feasible method for the comprehensive approach, and change in resistant status of a DBM population might be reflected through monitoring the elevation or amplification of a specific esterase isozyme of the larvae.

Amplification and absence of Est 9 in larval zymograms

Amplified and deprived Est 9 were found in the larval zymograms of ST12RR, ST26 and ST10 strains. Amplification of these isozymes in ST12RR progenies was, however, not accomplished by strengthening the malathion resistance in these larvae, and larvae that had only Est 9 enhanced were not tolerant to the dose of 66 μg of malathion. Intrabreeding of ST10 moths for only one generation ended with absence of Est 9 in zymograms of some larvae of the same brood. Absence of Est 9 was usually accomplished by expression of Est 8 in the zymogram. In some cases both Est 8 and Est 9 were diminished in the zymogram. Any brood of progenies that had either Est 8 or Est 9p enhanced would have sister-larvae being resistant to malathion. It is interesting to note that enhancement of Est 9p was very often found in ST10 strain, but not so often in ST26 or ST12RR strains, and was rarely found in ST12SS strain. Sequentially intrabreeding of ST12SS to second generation also bestowed upon the progenies very dense Est 9 bands in larval zymograms. In fact, the density of one-twelfth of Est 9 band of ST12SS larvae was even denser than that of one-third of the same isozyme band of the nonintrabred ST10 larvae. Nevertheless, this amplified Est 9 was as susceptible to paraoxon as any of the

Est 9 found in susceptible larvae. Amplification of Est 4 was found in most of the ST12-intrabred progenies, but was occasionally found in ST26 or ST10-intrabred progenies. In ST12 strain, amplification of Est 4 in zymograms was accomplished through elevation of other esterase isozymes. This phenomenon was, nevertheless, not found in the larvae of other strains. These results demonstrate that regulatory mechanisms for gene expression were involved in DBM resistance. Although we know that hydrolyases in Est 4 band could split malathion, but not malaoxon in vitro (Maa unpublished data), correlation of enhanced Est 4 and malathion resistance in ST12 progenies is still under investigation.

It seems that the frequency of Est 9p was indeed associated with malathion resistance in the DBM. The unknown mechanism(s) of regulation in expressing Est 4, Est 8 and Est 9p in mixed phases of diminishing, deprivation, elevation and amplification, was possibly involved in gene transposition in the DBM, since conventional rules of genetics seem not able to rule out the phenomenon observed in these experimental results.

Frequency of isozyme and resistance monitoring

Frequency of Est 9p in zymograms of 23 larvae of each of 17 DBM populations collected from different vegetable gardens around Taiwan, were plotted against the LD_{50} of the larvae of each of 17 DBM populations to four insecticides according to Cheng (1981) (Table 4). We also plotted the frequency of Est 9p against the LD_{50} of larvae to malathion. The results of analysis show that the correlation between frequency of Est 9p and the resistance of DBM larvae to insecticides was significant for malathion ($P < .01$), mevinphos ($P < .05$) and fenvalerate ($P < .05$), but not significant for carbofuran and permethrin. It seems that Est 9p alone could be used as a protein indicator for monitoring the resistance of DBM to malathion, mevinphos and fenvalerate. Chen (1985), however, found little common mechanism between OP compounds and synthetic pyrethroid resistance in fenvalerate-selected strains of DBM, and larvae of this DBM strain had high levels of cross-resistance to permethrin and carbofuran. On the other hand, Cheng (1986) reported that cross-resistance between OP compounds and synthetic pyrethroids was detected, and carbofuran resistance was independent of that of other insecticides. Cheng et al. (1986) emphasized that resistance of DBM to OP insecticides is not associated with mixed function oxidases (MFO), and synthetic pyrethroids and carbamates are chiefly detoxicated by MFO in DBM larvae. They also pointed out that MFO for synthetic pyrethroid was different from that for carbofuran. In Japan, Hama (1989) found that the Yokato population, which was susceptible to pyrethroid and resistant to OP compounds, exhibited a high resistance to DDT. The resistance levels of populations to OP compounds and carbamate insecticides were quite different. Noppun et al. (1986) found that the larvae of Okinawa strain gained high levels of resistance to phenthoate after eight selections during nine generations, and selection for resistance with fenvalerate in Okinawa strain was limited after 16 selection treatments for 23 generations. Reduced cuticular penetration was found as a major resistance mechanism in phenthoate-resistant DBM, and fenvalerate-resistant DBM strain respectively (Noppun et al. 1987, 1989). Miyata et al. (1986) suggested that the cross-resistance spectrum of DBM to various categories of insecticides is different between populations and insecticide resistance selection. Almost all of the resistant strains mentioned above were obtained by insecticide resistance selection, and increased detoxification enzyme, MFO or penetration factors were of most concern in these studies.

An important and interesting study on reversion of resistance of a pyrethroid-resistant strain of DBM from Thailand has been carried out by Motoyama and his co-workers (see elsewhere in this volume). They found some interesting mechanisms in the resistance. The heavily selected fenvalerate-resistant strain would spontaneously decrease 50% of resistance in every generation under relaxation of the selection pressure. The reversion of resistance was associated with the loss of reduced cuticular penetration and increased detoxification. The revertant maintained the third resistance mechanism: insensitivity of the target organ to insecticide. Another interesting fact Motoyama and co-workers found was that the fenvalerate resistance of the revertant larvae

Table 4. Correlation between the frequency of Est 9 of the DBM larvae and LC_{50} or LD_{50} of the larvae from different DBM populations.

DBM pop.	Est 9p freq.	Log values of LC_{50} of 4 insecticides				LD_{50} of malathion
		Fenvalerate	Carbofuran	Mevinphos	Permethrin	
ST	.92	3.88	3.14	2.53	3.67	2.29
SH	.70	2.72	2.45	1.95	2.52	2.24
TY	.25	3.07	2.33	2.04	2.75	1.71
CP	.92	3.52	2.70	2.41	2.96	1.94
ML	.65	3.28	2.37	2.45	2.88	2.20
TC	.54	3.66	2.64	2.48	3.10	2.31
TT	.86	3.39	2.40	2.43	3.06	2.31
HL	.71	3.72	2.67	2.61	3.60	2.32
PT	.96	3.99	2.53	2.52	3.41	2.34
LC	.96	3.76	2.92	2.84	3.58	2.27
LY	.83	4.00	2.87	2.68	3.67	2.60
PC	.70	3.64	2.21	2.40	3.10	2.47
HH	.83	3.58	2.41	2.51	3.70	2.40
HY	.79	3.98	2.55	2.29	3.35	2.34
KS	.71	3.50	2.54	2.35	3.09	2.31
PL	.85	3.45	2.45	2.42	2.95	2.27
MH	.88	3.66	2.32	2.28	3.13	2.23
CA	.67	–	–	–	–	2.26
IL	.79	–	–	–	–	2.23
YP	.75	–	–	–	–	2.22
SA	.61	–	–	–	–	1.99
GF	.25	–	–	–	–	1.98
Ksh	.58	–	–	–	–	1.94
KL	.79	–	–	–	–	1.91

LC data were transcribed from Cheng (1981). Sample size = 17 for carbofuran, fenvalerate, mevinphos and permethrin, and 24 for malathion (Maa et al. unpublished data). Linear regression assay: malathion: $Y = 1.83 + .006X$ r = .500 (<P.01)
Fenvalerate: $Y = 2.51 + .01X$ r = .5917 (<P.05)
Carbofuran: $Y = 2.20 + .005X$ r = .3613 not significant
Mevinphos: $Y = 1.97 + .006X$ r = .4936 (<P.05)
Permethrin: $Y = 2.62 + .008X$ r = .4091 not significant.

could be restored by just one selection pressure with fenvalerate or even with malathion. They thus concluded that there was an unknown factor(s) necessary to maintain the insecticide resistance in DBM, which cannot be explained by the conventional preadaptation theory. Since no insecticide was ever applied, as selection pressure to ST strains, it is reasonable to speculate that the reduced cuticular penetration and the increased detoxification should be excluded from the resistance mechanism of ST larvae to malathion. It is likely that esterase activity and insensitivity of the target site play an important role in resistance of ST strain to malathion. The elevation of Est 8/Est 9p was negatively associated with rate of aberrational growth of the treated larvae. The mechanism that regulated the amplification of Est 4 and 9 which influenced the expression of Est 8/Est 9p in different ST strains was possibly involved in the resistance restoring mechanism found in the revertant DBM in the study by Motoyama. Gene transposition thus might be involved in this restoring mechanism since the expression of Est8/Est9p in larval zymogram by one single intrabreeding of single-female mating was rarely found in ST12 strain.

Possible use of isozymes in resistance-grading

Since the esterase-isozyme patterns change with population, this isozyme-related phenomenon may be of use in determining the synchronization-resistance of field-collected DBM. As a result of our study, the isozyme patterns of Est 8/Est 9p showed a constant increase with resistance

of the population, suggesting that it could be a good diagnostic tool for resistance determination. Therefore a regression model for determining the resistance of DBM population by the relative abundance of Est 8/Est 9p was: Y = 1.83 + .006X for malathion, Y = 1.97 + .006X for mevinphos and Y = 2.51 + .01X for fenvalerate. The standard error of estimate can be minimized by increased replications (Table 4).

The conventional bioassay of LD_{50} of the insect to insecticide was very important in monitoring the insecticide resistance, and many techniques are used to determine the resistance of insects. Most of these techniques are only applicable to the insect with a sampling size of at least 100 individuals. Resistance management requires more effective techniques for detecting resistance in its early stages of development. The ideal technique for resistance-grading is still being sought. The advantages of using esterase isozyme analysis in resistance-grading are the ease of sample preparation, rapidity and accuracy. The total time required for sample preparation and resistance analysis would be about 1 day. The sample size can be fixed at 25 or reduced to 18, or increased to 50 as necessary. Unfortunately, this method can be undertaken only in the laboratory with the necessary equipment, but it still shows potential as a tool for resistance determination, at least to certain kinds of OP insecticides and possibly other synthetic pyrethroids.

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Resistance of Diamondback Moth to Insect Growth Regulators

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Abstract

Susceptibility of diamondback moth *Plutella xylostella* (L.) from Thailand to various insecticides was determined. Results showed that the diamondback moth was resistant to synthetic pyrethroids, organophosphorus insecticides, carbamates, as benzoylphenylurea well as insect growth regulators such as chlorfluazuron, diflubenzuron, hexaflumron, PH 70-23, NK-081 and NI-18. The degree of resistance to the last group varied widely among the chemical groups. The insect was sensitive to *Bacillus thuringiensis* Berliner, abamectin, NC-176 and juvenile hormone mimic. An addition of piperonyl butoxide to benzoylphenylurea insecticides resulted in no synergism, indicating that the resistance mechanism of this strain does not include microsomal oxidation of insecticides. In order to determine the mode of inheritance of insect growth regulator resistance, we conducted crossing and reciprocal tests using resistant and susceptible strains. The gene responsible for resistance seems to be completely recessive because the dose-mortality regression curve of F_1 progeny coincided with the susceptible strain. The result of backcrosses suggested that the inheritance of resistance was monofactorial. Reciprocal crossing test results showed that there was no sex linkage in this inheritance. During 40 generations of rearing without insecticide pressure no recovery of sensitivity to insect growth regulators was observed. Insect growth regulator-resistant strains of the diamondback moth emerged in 1989 at Kagoshima and Okinawa in Japan. The Kagoshima strain showed low levels of resistance to insect growth regulators.

Introduction

Diamondback moth (DBM) *Plutella xylostella* (L.) (Lepidoptera:Yponomeutidae) has become the most important insect pest of crucifers, and has developed resistance to organophosphorus insecticides (Noppun et al. 1986), carbamates (Sun et al. 1978), synthetic pyrethroids (Liu et al. 1981, 1982; Hama 1986; Horikiri et al. 1987, Makino et al. 1985, Noppun et al. 1986), *Bacillus thuringiensis* Berliner preparations, and others. In Southeast Asian countries, including Thailand, this increased resistance to insecticides is causing major problems for the growers.

Benzoylphenylurea insect growth regulators (IGR) with superior toxicity to the resistant DBM have been developed. However, Perng (1987), Perng et al. (1988), Kohyama et al. (1989), and Lin et al. (1989) found resistance in DBM even to these IGRs.

Susceptibility tests on various IGRs were performed on the DBM collected in Thailand. The results showed that the DBM had developed resistance to IGRs. To clarify the features of the resistance to IGRs, we carried out a number of investigations on the susceptibility, synergists, genetic mechanisms responsible for resistance, stability of IGR resistance, and others.

Materials and Methods

Test insect

The following four strains of DBM were used in this study: a BBT strain collected at Bang Bua Thong near Bangkok in January 1988; a BK strain collected at Bangkhae in the suburbs of Bangkok in January 1988, an MZB strain collected at Mizobe Kagoshima, Japan, in April 1990, and a susceptible strain maintained at Agricultural Technical Center, Zen-Noh, Japan.

The BBT and BK strains are presumed to have been exposed to DDT, malathion, dichlorvos, methomyl, carbofuran, fenvalerate, permethrin, cypermethrin, teflubenzuron, and since 1985 chlorfluazuron and *B. thuringiensis*. MZB strain was exposed to the plant foot application of benfuracarb, acephate granules, fluvalinate wettable powder and a *B. thuringiensis* preparation. However, the toxicity of these insecticides to MZB strain has been decreasing in recent years. For this reason, when chlorfluazuron was registered in October 1988, it was supposed to have been used for its superior efficacy.

In our tests, the S strain was designated the standard strain. The test insects were the 3rd instar larvae reared without IGR pressure at 25±2°C, 16L:8D (Koshihara and Yamada 1978). The test of juvenile hormone mimic (JHM) used 4th instar larvae.

Test chemicals

The following insecticides were used in this study: IGRs: chlorfluazuron 5% emulsifiable concentration (EC), diflubenzuron 23.5% wettable powder (WP), hexaflumron 5%EC, PH 70-23 25% liquid formulation (L), NK-081 5%EC, NI-18 2.5%EC and synthetic pyrethroids; fenvalerate 96.3% technical (Tech.), permethrin 96.4% technical and ethofenprox 96% technical. Carbamates: carbofuran 97.3% technical and methomyl 91% technical. Organophosphorus insecticides: dichlorvos 99% technical, dimethylvinphos 96% technical, trichlorfon 99.7% technical, vamidothion 54.2% technical, fenitrothion 96.5% technical, diazinon 96% technical, salithion 95.5% technical, pirimiphos methyl 91.5% technical, EPN 92.7% technical, phenthoate technical, prothiophos 94.1% technical, malathion 95% technical, acephate 97% technical and *B. thuringiensis* preparations; Toarrow-CT 7%WP, Dipel 10%WP and cartap 50% soluble powder (SP), JHM NC-184, abamectin (MK-936) and NC-176.

These formulated chemicals were diluted with deionized water, and the spreader Neo-Esterin was added. Technical grades of chemicals were dissolved in a small amount of acetone before dilution. Synergist tests were carried out by adding 100 ppm piperonyl butoxide equivalent to each chemical solution.

Susceptibility tests

Each test was conducted by dipping a cabbage leaf in an aqueous solution of chemicals for 1 min. Five to seven concentrations of each chemical with three replications were tested. After being air-dried, the leaf was put in the cup (diameter 9 cm, height 5 cm) and 10 DBM larvae were released. The larvae were maintained in a chamber controlled at 25±2°C, 16L:8D. Mortality and inhibition of adult eclosion were recorded as follows: 5 days after treatments on IGRs for mortality, 7 days after treatments on JHM for inhibitor of adult eclosion and 2 days after treatments of other chemicals for mortality.

The results were analyzed by the probit method (Bliss 1935) on connected mortality (Abbott 1925). LC_{50} values were calculated.

Crossing tests

Pupae from the BBT, S strain and their F_1 were put in a glass tube 8 mm diameter and 50 mm high. A day after adult eclosion, 20 male and female adults from the respective strains were put into a 15 cm diameter cup for the reciprocal crossing, back-crossing between the BBT strain and F_1 that sprang from the reciprocal crossing, and the crossing of F_2. The susceptibility test in this crossing was carried out with the leaf dipping method using 3rd instar larvae.

The mode of inheritance of DBM resistance to IGRs was investigated using degree of dominance (D) and chlorfluazuron concentration-mortality curves. The degrees of dominance were calculated using Stone's (1968) formula.

Results and Discussion

Susceptibility of DBM to various IGRs

Susceptibility of BBT and BK strains of DBM to various IGRs decreased, but the degree of resistance varied widely among the chemical groups belonging to IGRs (Table 1). For example, resistance ratio (RR) of PH 70-23 was 49, 080-fold on BBT strain, while RR of NI-18 was 8 and that of hexaflumron 12. Among organophosphorus compounds, it is known that cross-resistance is usually not detected between phenthoate and dimethylvinphos. It is also assumed that resistance of IGRs has a tendency similar to the organophosphorus resistance. Therefore, IGR resistance is presumed to have no cross-resistance, largely due to their unique mode of action. Future studies on the IGRs mode of action should clarify this.

DBM from chlorfluazuron-used area showed less susceptibility to other IGRs. A certain difference in the susceptibility to BBT and BK strains was noticeable in the chlorfluazuron, but it remained quite similar to the other chemicals.

Table 1. Susceptibility to several chitin synthesis inhibitors of the larvae of susceptible (S) and resistant (BBT, BK) strains of DBM.

Insecticide	BBT-strain		BK-strain		S-strain
	LC_{50} (ppm)	RR[a]	LC_{50} (ppm)	RR[a]	LC_{50} (ppm)
chlorfluazuron 5%EC	13	130	1.4	14	0.1
diflubenzuron 23.5%WP	6910	28	6544	26	248
hexaflumron 5%EC	1.8	12	–[b]	–	0.15
PH 70-23 25%L	18945	49080	–	–	0.39
NK-081 5%EC	160	5517	206	7103	0.029
NI-18 2.5%EC	1.2	8	2.0	13	0.16

[a]RR: Resistance Ratio, (LC_{50} of resistant strains/LC_{50} of susceptible strain). [b]–: Not available.

Susceptibility of DBM to various insecticides

Synthetic pyrethroids and carbamates have not been used recently in Thailand, however the BBT and BK strains showed decreased susceptibility. DBM also showed a lowered susceptibility to organophosphorus compounds, but dimethylvinphos, pirimiphos methyl and phenthoate which have higher toxicity to DBM, were still effective on these strains. These results are similar to other recent reports. The relationship between structure and activity of three organophosphorus compounds was carefully investigated. The three insecticides belonged to three classes of organophosphorus compounds, indicating no structural similarity to each other. A difference in the susceptibility to BBT and BK strains was noticeable in phenthoate, but it remained similar in the other chemicals. It is not clear whether cartap was used in the area investigated, however, a certain lowering of susceptibility was observed indicating sufficient efficacy with the conventional application (Table 2). Among the two *B. thuringiensis* preparations, Dipel showed a lower susceptibility than Toarrow-CT. It is reported that Thuricide was used in Thailand, so it is necessary to look into the cause and effect in more detail.

These results confirmed that the BBT and BK strains collected in Thailand showed a multiple resistance to various insecticides.

Table 2. Susceptibility to several groups of insecticides of the larvae of susceptible (S) and resistant (BBT, BK) strains of DBM.

Insecticide	Formulation	BBT-strain		BK-strain		S-strain
		LC_{50} (ppm)	RR[a]	LC_{50} (ppm)	RR[a]	LC_{50} (ppm)
fenvalerate	96.3%Technical	190	100	186	98	1.9
permethrin	96.4%Technical	122	37	120	36	3.3
ethofenprop	96%Technical	421	13	435	13	33
carbofuran	97.3%Technical	177	12	219	15	15
methomyl	91%Technical	1907	10	1724	9	183
dichlorvos	99%Technical	>1000	ND[d]	≤1000	ND	60
dimethylvinphos	96%Technical	133	27	147	30	4.9
trichlorfon	99.7%Technical	>1000	ND	>1000	ND	178
vamidothion	54.2%Technical	>1000	ND	≤1000	ND	9503
fenitrothion	96.5%Technical	>1000	ND	>1000	ND	196
diazinon	96%Technical	>1000	ND	≤1000	ND	15
salithion	95.5%Technical	>1000	ND	<1000	ND	20
pirimiphos methyl	91.5%Technical	138	24	89	16	5.7
EPN	92.7%Technical	>1000	ND	>1000	ND	693
phenthoate	Technical	88	33	303	112	2.7
prothiophos	94.1%Technical	812	677	<1000	ND	1.2
malathion	95%Technical	>1000	ND	>1000	ND	251
acephate	97%Technical	>1000	ND	<1000	ND	37
cartap	50%SP	113	10	121	11	11
Toarrow-CT[b]	7%WP	3	3	2.9	3	0.97
Dipel[b]	10%WP	18.1	10	–[c]	–	1.77

[a]RR: Resistance Ratio (LC_{50} of resistant strains/LC_{50} of susceptible strain). [b]Trade marks valid in Japan. [c]Not available. [d]ND: Not determined.

The effect of new insecticides

Abamectin 2%WP and NC-176 10%EC showed extremely high activity against S and BBT strains. The RR for abamectin was 11, and that for NC-176, 3.4, and the concentration of conventional application was considered effective.

JHM of NC-184 showed extremely high activity against S strain. However, the inhibition concentrations for over 80% adult eclosion was 10 ppm in BBT as against 1 ppm in S strain.

This suggests that both IGRs and JHM acted similarly in the metamorphosis, resulting in the possibility of cross resistance between two. However, since NC-184 is highly active, it should work effectively at conventional concentrations.

The NC-184 is slow to produce effect because of its activity only at pupation and adult eclosion stages. For this reason, the feeding should continue until the effect begins. Regardless of its unique activity, its practical application will necessitate further research.

Synergistic effect

No synergism with addition of up to 100 ppm piperonyl butoxide (PB) was observed in a chlorfluazuron-resistant DBM strain having an RR of 405. Perng et al. (1988) have reported that PB has synergism (SR:8.8) to teflubenzuron in the teflubenzuron-resistant DBM (RR:12). In our test, however, PB did not show any synergism to chlorfluazuron in DBM having a resistance of 405. This suggests that the microsomal oxidases did not play any major role in the mechanism of chlorfluazuron resistance.

It will therefore be necessary to study the treatment method of PB and its concentration, and use of other inhibitors. Through a detailed study of synergists, some clues may be obtained about the mechanism of IGR resistance.

The mode of inheritance in IGR resistance

From the crossing tests between S and BBT strains, it was observed that the susceptibility of F_1 progeny coincided with that of S strain. Furthermore, judging from the degree of dominance (D = -1), it was suggested that the mode of inheritance in the IGR resistance was completely recessive. Since no difference in susceptibility in the reciprocal crossing tests was noticed in each F_1 progeny, it was confirmed that the IGR resistance is not sex linked. Also, from the dose-mortality curves of F_2 and back-crossing, it was confirmed that the inheritance originated from the monofactorial major gene (Fig. 1).

Recovery of susceptibility in absence of IGR pressure

An investigation was done on the fluctuation of susceptibility to chlorfluazuron during 40 generations. Every LC_{50} value remained within 95% confidence limit to chlorfluazuron, and almost no sign of recovery in the susceptibility was observed (Fig 2).

The gene frequency in the population, or the relative merits of gene, is an important factor for development of IGR resistance. Such a factor has, however, almost nothing to do with the possibility for recovery of susceptibility that was once reduced. Hardy-Weinberg's law stipulates that the gene frequency in the population and the ratio of gene type do not vary even during generations. The law is supposedly applicable under the rearing conditions we used.

The recovery of susceptibility in a resistant population is presumably attributed to the case where the existential frequency of susceptible gene goes up in the population. The recovery of susceptibility is a result of inferior intrinsic rate of natural increase and inferior fitness, and the recovery is also supposedly caused by blended genes of foreign populations or by obtaining the resistant gene. Furthermore, the recovery of susceptibility largely depends upon whether the population is homogeneous or heterogeneous with regard to resistance.

From the above, we assumed that the BBT strain remained no less able for the above intrinsic rate of natural increase and fitness, regardless of the resistant gene carried by the BBT. Also it was suggested that the population in question was possibly a homogeneous group regarding resistance.

IGR-resistant DBM in Kagoshima, Japan

A problem arose in Japan in 1989 when DBM's susceptibility to IGR suddenly decreased. To study this, the susceptibility to various chitin synthesis inhibitors was examined. The MZB

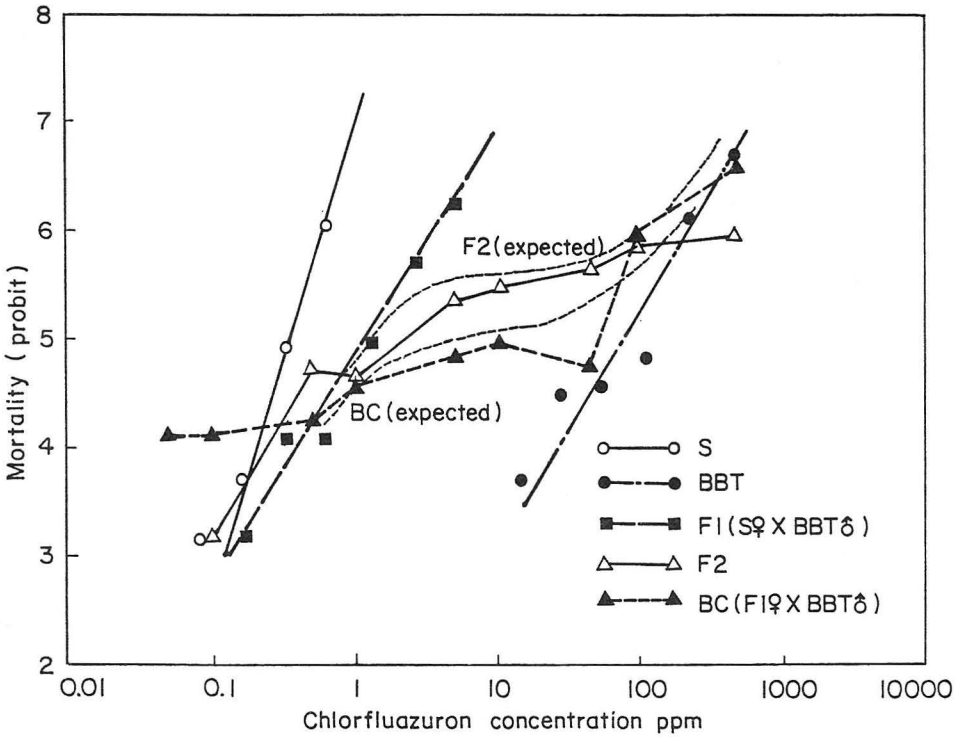

Fig. 1. Dose-mortality regression lines for chlorfluazuron against susceptible (S), resistant (BBT) strain and crosses progeny of the DBM.

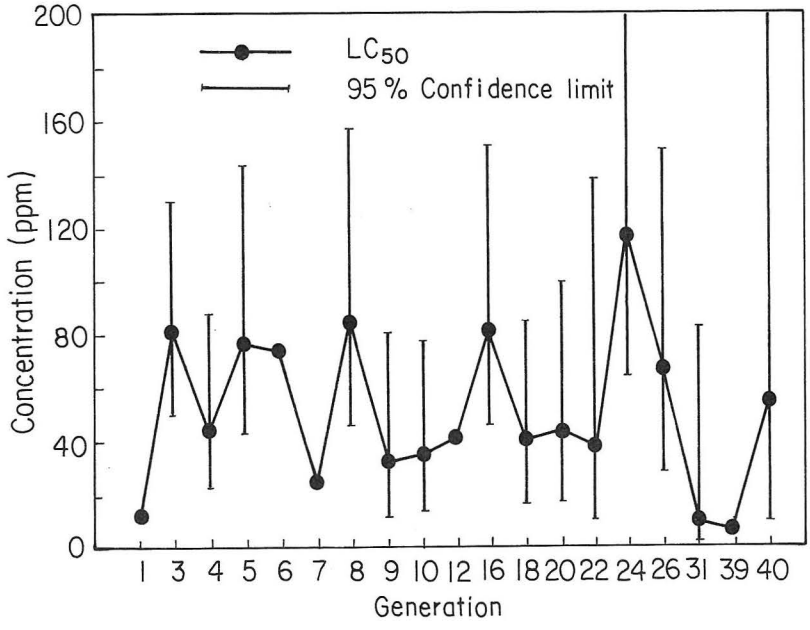

Fig. 2. Stability of resistance to chlorfluazuron of the resistant (BBT) strain of DBM upon noninsecticide pressure.

strain had a resistance ratio of 24.3 to chlorfluazuron, indicating a lowering trend of susceptibility to other IGRs as well. The resistance level in the MZB strain was below that of the BBT and BK strains. The MZB strain, however, showed a similar level of lowered susceptibility to other IGRs (Table 3).

Thus, although the IGRs are considered to be highly effective insecticides, as seen in the MZB strain, the resistance to these chemicals is an unavoidable problem.

Our study has given us new insight into IGR resistance. Although the IGR resistance was completely recessive it was supposed to develop in the parallel with the increasing insecticide pressure. As there are currently few promising replacements, some insecticides have to be used urgently to curb resistance. Based upon this information, a proper method of DBM control is urgently needed. From our test results, some conventional insecticides were found to have useful activities and rotational use of these insecticides would help avoid or delay the IGR resistance.

It may also be necessary to search for some mixture in the application of synergism. More study is needed to clarify the mechanism of DBM resistance to IGRs.

Pimprikar and Georghiou (1979, 1982) pointed out that the resistance mechanism of diflubenzuron in the housefly is collectively associated with the reduced chitin synthesis, effect of reduced cuticular penetration, increased metabolism and the rapid excretion of the chemical. In the case of DBM, the time shift for chitin synthesis is inducing the resistance, as reported by Kurihara et al. (1990). Also Perng et al. (1988) reported detoxification by microsomal oxidation, suggesting that several factors play a part in the resistance.

Table 3. Susceptibility to several chitin synthesis inhibitors of the larvae of susceptible (S) and low level-resistant (MZB) strains of DBM.

Insecticide	LC_{50} ppm	MZB-strain (95% CL)[a]	Slope	RR[b]	S-strain[c] LC_{50} ppm
chlorfluazuron 5%EC	2.43	(1.33-4.43)	1.67	24.3	0.1
diflubenzuron 23.5% WP	2285	(ND[d])	1.34	9.2	248
hexaflumron 5% EC	2.53	(1.70-4.06)	1.87	16.9	0.15
PH 70-23 25%L	9.53	(2.10-44.3)	1.35	24.4	0.39
NK-081 5%EC	0.49	(0.17-1.38)	1.01	16.9	0.029
NI-18 2.5% EC	1.06	(0.56-2.29)	2.97	6.6	0.16

[a]95% CL: 95% Confidence Limit. [b]RR: Resistance Ratio, (LC_{50} of resistant strains/LC_{50} of susceptible strain). [c]LC_{50} of S-strain quoted from another source. [d]ND: Not determined.

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Resistance to Acylurea Compounds in Diamondback Moth

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Abstract

The toxicity of three acylurea insect growth regulators, chlorfluazuron (Atabron),teflubenzuron (Nomolt) and diflubenzuron (Dimilin), and the macrocyclic lactone, abamectin, was assessed against a laboratory, susceptible (FS) strain and a field (Cameron Highlands, Malaysia 1988) strain of the diamondback moth, *Plutella xylostella* (L.) using a leaf-dip bioassay with second instar larvae. Based on LC_{50} values, the order of toxicity against the FS and Cameron Highlands (CH) populations was: abamectin > chlorfluazuron = teflubenzuron >> diflubenzuron. The CH population ($F_{6/7}$ from collection) was found to be 12.6-, 6.7-, 6.4- and 2.3-fold less sensitive to diflubenzuron, teflubenzuron, chlorfluazuron and abamectin, respectively, when compared with the FS strain. Separate bioassays for chlorfluazuron and teflubenzuron with a second field strain (Serdang, Malaysia 1988) gave similar results when compared with CH, and in a concurrent assay the Serdang population (F_{1-3}) was 5.1-fold less sensitive to chlorfluazuron compared with the FS strain. Selection of subpopulations of the CH strain with chlorfluazuron (ATA-SEL) and teflubenzuron (NOM-SEL) for six generations in the laboratory, increased LC_{50} resistance ratios to 112- and 312-fold respectively compared with the FS strain. Marked cross-resistance was also demonstrated between chlorfluazuron and teflubenzuron. However, the ATA-SEL and NOM-SEL subpopulations showed no evidence of cross-resistance to diflubenzuron and abamectin. Subsequent experiments also showed relatively little cross-resistance to two other acylureas, flufenoxuron (Cascade) and hexaflumuron (Consult). Unlike the FS strain, ATA-SEL and NOM-SEL insects pretreated with the synergists piperonyl butoxide or S, S, S-tributyl phosphorotrithioate showed increased susceptibility to chlorfluazuron and teflubenzuron respectively; suggesting microsomal monooxygenases and esterases may be involved in resistance. The present results are discussed in relation to studies on other populations of diamondback moth where similarly variable, but different patterns of cross-resistance between acylureas have been reported. A common feature in all studies on this species to date is the apparent lack of cross-resistance between acylureas and other groups of insecticides.

Introduction

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), is a major and cosmopolitan pest of cruciferous crops. In Malaysia, for example, it is the principal pest of both upland (e.g. Cameron Highlands) and lowland vegetable-producing regions (Ooi 1986).

The DBM has proved increasingly difficult to control due largely to its marked ability for development of resistance to insecticides, most notably in Southeast Asia and the Far East (Talekar and Griggs 1986; Tang et al. 1988). There are more recent reports of resistance to insecticides in North and Central American populations of DBM (Georghiou 1989; Shelton and Wyman 1990; Andrews et al. 1990). Field resistance to all major groups of insecticides, including organophosphorus, carbamates and pyrethroids, has now been reported among various populations of this species (Cheng 1988, 1990; Sun 1990).

In the past few years, evidence of field resistance to the acylurea insect growth regulators (Rushtapakornchai and Vattanatangum 1986; Miyata et al. 1988; Rushtapakornchai et al. 1988; Cheng 1988; Perng et al. 1988; Kobayashi et al. 1990; Syed 1990) and to products based on the insecticidal spore-crystal protein complex of certain *Bacillus thuringiensis* Berliner strains (Rushtapakornchai et al. 1988; Miyata et al. 1988; Tabashnik et al. 1990, 1991; Hama 1990; Syed 1990) has been reported in DBM. There is also one report of low level cross-resistance to the novel, macrocyclic lactone insecticide, avermectin B1, in a multiple insecticide-resistant population of DBM collected in Thailand in 1982 (Abro et al. 1988).

The development of resistance to *B. thuringiensis* and acylureas is of particular concern as these products are generally rated harmless to beneficial arthropods, including natural enemies (Flexner et al. 1986; Oomen and Wiegers 1984; Perugia et al. 1986; Anderson et al. 1986a; Scheletes et al. 1988; Fisher and Holtmann 1989; Fauziah 1990). Such compounds are thus of particular value in integrated pest management (IPM) of DBM and other insect pests.

In the present work, a population of DBM was collected from the Cameron Highlands, Malaysia, in March 1988 for study in the U.K. Since the acylurea, diflubenzuron, had been widely used against DBM in Malaysia for at least 2 years (Ooi 1986), and teflubenzuron and chlorfluazuron had also been applied (Syed 1990), whether there was field resistance to these compounds and cross-resistance to other acylureas or to the chemically unrelated insecticide, abamectin (avermectin B1), was of particular interest. As part of a collaborative program with the Universiti Pertanian Malaysia, a second field population was later collected from a lowland region of Malaysia for comparative studies.

Materials and Methods

Test insects

Three strains of DBM were studied: a laboratory, insecticide susceptible strain (FS; Liu et al. 1984) maintained in culture in the U.K. and at Universiti Pertanian Malaysia (UPM); a field strain collected in the Cameron Highlands (CH), Malaysia, in March 1988 and subsequently maintained in the U.K.; a field strain collected in a lowland area (Serdang) of Malaysia from November 1988 and maintained at UPM.

In the U.K., the FS and CH strains were cultured and tested on Chinese cabbage (*Brassica campestris* var. *chinensis* cv Pe Tsai) at 20±2°C and 63±3% RH under a 16L:8D cycle. In Malaysia, the FS and Serdang (SER) strains were cultured and tested on Sawi (*Brassica juncea*) at 28±2°C and 70-80% RH under natural light conditions. Larval instars of DBM were identified by the width of the head capsule (Fauziah 1990).

Chemicals

The compounds tested were: chlorfluazuron (Atabron), 5% (w/v) emulsifiable concentrate (EC); teflubenzuron (Nomolt), 15% (w/v) suspension concentrate (SC); diflubenzuron (Dimilin), 25% (w/w) wettable powder (WP); hexaflumuron (Consult), 5% (w/v) EC; flufenoxuron (Cascade), 10% (w/v) EC; and abamectin (avermectin B1), 1.8% (w/v) EC. Test solutions of pesticides were freshly prepared for each assay in distilled water with Triton X-100 (50 ppm) as an additional surfactant. Stock solutions of technical grade piperonyl butoxide (PB; 90% w/v)

and S,S,S-tributyl phosphorotrithioate (DEF; 99.4% w/v) were prepared in ethyl methyl ketone (EMK) and stored at -20°C.

Residual Ingestion Bioassays

Residual bioassays were used to assess the toxicity of each test chemical. In the U.K., the FS and CH strains were assayed on leaf discs cut from the middle leaves of 14-day-old Chinese cabbage. Each leaf disc was immersed in the test solution for 10 sec, then drained against filter paper and allowed to dry for approximately 1 hour at ambient laboratory temperature (about 20°C). The leaf discs were then placed on a filter paper in individual, plastic petri dishes (5 cm diameter). Control leaf discs were dipped in distilled water with Triton X-100 (50 ppm). Five, 2-day-old second instar DBM larvae were placed on each leaf disc. After 5 days, the treated leaf discs were replaced by fresh, untreated Chinese cabbage leaves, which were themselves replaced at regular intervals. Larval mortality was assessed up to 17 days from initial exposure to pesticide, after which pupation had usually occurred among the survivors in all treatments. For each pesticide, 6-7 treatments were tested (50 insects per treatment).

In Malaysia, the FS and SER strains were bioassayed in a similar way on leaf discs cut from the middle leaves of 8-week-old Sawi Plants. After 2 days, the treated leaf discs were replaced by fresh, untreated Sawi leaves. Larval mortality was assessed up to 4 days from initial exposure to pesticide, after which pupation had usually occurred. For each pesticide, a minimum of 6 treatments was used (20 insects per treatment). Each experiment was replicated six times and the data combined to estimate LC_{50} values.

Selection Experiments

Two subpopulations of the CH strain were selected in the U.K. against chlorfluazuron (ATA-SEL) and teflubenzuron (NOM-SEL) respectively for six consecutive generations (F_{6-11}). Second instar larvae of the F_6 generation were exposed to leaf discs of Chinese cabbage dipped in concentrations of pesticide estimated to give approximately 50% mortality in concurrent bioassays (see above). The concentration of pesticide used to select each subsequent generation (100-1080 insects per selection) was based on the mortality obtained in the previous assay (Fauziah 1990). The selected subpopulations were bioassayed on leaf discs at generations F_{12-16} to estimate the degree of resistance or cross-resistance to different pesticides (6-7 treatments per pesticide, 50 insects per treatment).

Combined topical/residual bioassays with synergists

Test solutions of PB or DEF in EMK (Fauziah 1990), or EMK alone were applied using an Arnold microapplicator (Burkard Manufacturing Co. Ltd., U.K.) to 2-day-old second instar larvae (FS, ATA-SEL and NOM-SEL). After 1 hour, batches of five larvae were placed on leaf discs of Chinese cabbage treated with chlorfluazuron or teflubenzuron and bioassayed as described above. Controls were treated with PB or DEF in EMK, or EMK alone, and placed on leaf discs dipped in Triton X-100 (50 ppm). For each pesticide, 6-8 treatments were used (50 insects per treatment).

Statistical analysis

Estimates of LC/LD_{50} values and their 95% fiducial limits (FL) were obtained using a probit program (S103, Statistical Research Service, Canada Department of Agriculture, unpublished) based on Finney (1971). When the regression lines for two treatments were not significantly ($P > 0.05$) different (equal slopes), the relative toxicity or resistance ratio was estimated using LC_{50} data from joint probit line analyses. Due to the inherent variabiity of bioassays, LC/LD_{50} values were compared at the 1% significance level using individual 95% FL for two parameters.

Results and Discussion

At the LC_{50} level, abamectin was found to be significantly ($P < 0.01$) more toxic than the acylureas tested; being approximately 15-, 19- and 350,000-fold more potent than chlorfluazuron, teflubenzuron and diflubenzuron, respectively, against the FS strain (Table 1). For the CH strain, the order of toxicity was the same but the differences between the compounds were somewhat greater. However, within each strain, there was no significant ($P>0.01$) difference at the LC_{50} level between chlorfluazuron and teflubenzuron (Table 1). The latter two compounds have also been reported to show reasonably similar levels of toxicity in seven strains of DBM from Taiwan (Perng and Sun 1987). The toxicity of abamectin was similar to that reported against another laboratory strain of DBM (Abro et al. 1988) and this compound has been reported to give excellent control of DBM in trials conducted in the Cameron Highlands (Hong and Hong 1986).

Table 1. Relative toxicity of acylureas against a susceptible, laboratory strain (FS) and two field strains (Cameron Highlands and Serdang) of DBM in a leaf-dip bioassay with second instar larvae.[a]

Insecticide	Strain[b]	LC_{50} (ppm ai)	95% FL	Slope ± SE	RT[c]
Chlorfluazuron	FS	0.015	0.012-0.018	1.53 ± 0.09	6.4
	CH	0.093	0.076-0.115		
	FS	0.018	0.008-0.279	0.95 ± 0.09	5.1
	SER	0.092	0.058-0.126		
Teflubenzuron	FS	0.018	0.014-0.023	1.22 ± 0.07	6.7
	CH	0.122	0.095-0.159		
	SER	0.422	0.217-0.645	1.04 ± 0.10	–
Diflubenzuron	FS	338	227-510	0.67 ± 0.06	12.6
	CH	4257	2841-6785		
Abamectin	FS	0.0010	0.0009-0.0011	2.31 ± 0.12	2.3
	CH	0.0023	0.0019-0.0026		

[a]Assays at 20°C on Chinese cabbage (FS v CH) or at 28°C on Sawi (FS v SER): mortality at 17 days (FS v CH) or 4 days (FS or SER). [b]Cameron Highlands (CH) and Serdang populations assayed at F_6-F_7 and F_{1-3} generations from field collected respectively (data combined for probit analysis). [c]Relative toxicity (RT) ratios for LC_{50} values were obtained using a test for parallelism (equal slopes).

The greater insecticidal activity of second generation acylureas, such as chlorfluazuron and teflubenzuron, compared with diflubenzuron has been widely reported (Haga et al. 1987; Fisher and Holtmann 1989) and may be related in part to different rates of metabolic detoxication, transport and elimination (Ishaaya and Yablonski 1987; Clarke and Jewess 1990). However, the relatively poor performance of WP compared with EC formulations of acylureas may also be a factor, particle size being critical for the insecticidal activity of the WP formulation of diflubenzuron (Mulder and Gijswijt 1973).

Bioassays at UPM with the FS strain, conducted at a higher temperature but over a much shorter assessment period gave a very similar LC_{50} value for chlorfluazuron when compared with the UK population derived from a common stock (Table 1). This suggests that the assay system for such compounds is reasonably robust and since, as chitin synthesis inhibitors, acylureas act during moulting (Reynolds 1987) it is the developmental period during the bioassay which is the most important factor.

The bioassays for the SER strain gave very similar LC_{50} values for chlorfluazuron when compared with the CH strain, but teflubenzuron was significantly ($P<0.01$) less toxic (3.5-fold) against the SER strain compared with the CH strain (Table 1).

Comparisons at the LC_{50} level indicated that the CH strain was 2.3-, 6.4-, 6.7- and 12.6-fold less sensitive to abamectin, chlorfluazuron, teflubenzuron and diflubenzuron respectively when compared with the FS strain (Table 1), whereas the SER strain was 5.1-fold less sensitive to chlorfluazuron compared with the corresponding FS population (Table 1).

Although the above differences between strains could have been due to the greater overall fitness (vigor tolerance) of field compared with laboratory populations, the history of usage of acylureas in Malaysia (Syed 1990) suggested the occurrence of resistance to diflubenzuron and possibly the other compounds. Subsequent selection of the CH strain for six generations demonstrated that resistance to chlorfluazuron and teflubenzuron was present (Table 2).

Table 2. Development of resistance in subpopulations of Cameron Highlands (CH) strain of DBM selected with Atabron (ATA) or Nomolt (NOM) for six generations (F_6-F_{11}): leaf-dip bioassay with second instar larvae.[a]

Strain[b] (product)	LC_{50} (ppm ai)	95% FL	Slope ± SE[c]	RR1[d]	RR2[e]
(F6-7)					
Chlorfluazuron	0.095	0.078-0.114	1.71 ± 0.13a	6.4	–
Teflubenzuron	0.121	0.094-0.159	1.25 ± 0.10b	6.7	–
ATA (F12)					
Chlorfluazuron	1.64	1.15-2.56	0.83 ± 0.09c	112	17.3
NOM (F12)					
Teflubenzuron	5.67	3.67-8.93	0.61 ± 0.08c	312	46.8

[a]Assays at 20°C on Chinese cabbage: mortality assessed at day 17. [b]Unselected CH strain assayed at F_6 and F_7 generations from field collection (data combined for probit analysis). [c]Values with a common letter not significantly ($P>0.05$) different. [d]LC_{50} resistance ratio (RR1) compared with FS strain (Table 1). [e]LC_{50} resistance ratio (RR2) compared with unselected CH strain.

Note: The LC_{50} doses for diflubenzuron and abamectin (CH F_{6-7}) gave 72-92% mortality in the ATA and NOM-selected strains at F_{12} (Fauziah 1990).

The speed at which resistance was selected suggested that field levels of resistance may be much greater than implied by the initial laboratory work on the $F_{6/7}$ generations, where resistance could have declined markedly from collection. In fact, resistance to acylureas was reported to have curtailed their use in the Cameron Highlands during 1988, with up to a 36-fold resistance in F_{1-3} generations of DBM (Syed 1990).

Further studies have shown that resistance to acylureas is relatively unstable in the CH strain (Fauziah 1990). Similarly, Cheng (1990) observed that resistance to acylureas was unstable in various populations of DBM from Taiwan. In contrast, acylurea resistance in a population from Thailand was reported to be stable for over 40 generations (Kobayashi et al. 1990).

Although it was unlikely that the selection pressure for the ATA-SEL and NOM-SEL subpopulations was equal in the above experiment, subsequent cross-resistance studies (Table 3) clearly showed resistance to teflubenzuron was selected at a greater rate compared with chlorfluazuron irrespective of the selection agent used. The degree and rate of selection of resistance to acylureas achieved with the CH strain was markedly greater than that reported

for either a laboratory strain of DBM or a composite field strain collected from seven locations throughout Taiwan in 1987 (Perng et al. 1988).

In the latter study, selection with teflubenzuron for 20 generations or more resulted in only an 8- to 12-fold resistance and it was suggested that the field populations from Taiwan might not have the major resistance genes required for the high levels of resistance reported in Thailand. However, the following year a rapid development of resistance (30-fold) to teflubenzuron was reported following failure of this compound to control DBM in Taiwan (Sun 1988). In the present study, there was clear evidence for cross-resistance between teflubenzuron and chlorfluazuron (Table 3). Selection of various Taiwan populations of DBM with teflubenzuron showed no apparent cross-resistance to chlorfluazuron (Perng et al. 1988), and selection of resistance to chlorfluazuron in a population from Thailand conferred little or no cross-resistance to teflubenzuron (Fahmy and Miyata 1990).

However, selection of the CH strain with either teflubenzuron or chlorfluazuron for six generations had no apparent effect on the response to either diflubenzuron and abamectin (Table 3). There was also little or no cross-resistance between teflubenzuron or chlorfluazuron and two other acylureas, hexaflumuron and flufenoxuron, although interpretation of the results was complicated by the absence of baseline ($F_{6/7}$) data for these last two compounds against the CH strain (Table 3). Relatively low levels of resistance to hexaflumuron and diflubenzuron have also been reported in a field population of DBM from Thailand which showed much greater resistance to some other acylureas (Kobayashi et al. 1990).

Treatment of the standard susceptible (FS) strain with the synergist PB, an inhibitor of microsomal (mixed function) monooxygenases, or with DEF, an esterase inhibitor, had no significant ($P>0.01$) effect on the toxicity of chlorfluazuron or teflubenzuron (Table 4). However, with the ATA-SEL and NOM-SEL subpopulations both synergists significantly ($P<0.01$) increased the toxicity of chlorfluazuron and teflubenzuron respectively (Table 5). Although the levels of synergism achieved by PB or DEF individually (2.9 to 16.5-fold) were relatively small compared with the apparent levels of resistance (55- to 149-fold), the combined effects of microsomal oxidation and hydrolysis by esterases could make a substantial contribution to resistance in the CH strain. The involvement of esterase activity in the hydrolysis of

Table 3. Cross-resistance to acylureas in Atabron (ATA) and Nomolt (NOM)-selected (F_6-F_{11}) subpopulations of the Cameron Highlands (CH) strain of DBM: leaf-dip bioassay with second instar larvae.[a]

Strain (product)	LC_{50} (ppm ai)	95% FL	Slope ± SE	RR1[b]	RR2[c]
ATA-selected					
Teflubenzuron (F_{14})	8.28	5.14-13.3	0.76 ± 0.10	456	68.3
Hexaflumuron (F_{15})	0.447	0.271-0.726	0.81 ± 0.12	10.3	–
Flufenoxuron (F_{15})	0.118	0.062-3.474	0.61 ± 0.08	15.8	–
Chlorfluazuron (F_{16})	1.09	0.688-1.93	0.83 ± 0.10	74.7	11.4
NOM-selected					
Chlorfluazuron (F_{14})	0.887	0.398-4.474	0.37 ± 0.06	60.7	9.3
Hexaflumuron (F_{15})	0.182	0.079-0.366	0.54 ± 0.09	4.2	–
Flufenoxuron (F_{15})	0.107	0.057-0.222	0.55 ± 0.07	13.9	–
Teflubenzuron (F_{16})	2.73	1.65-4.53	0.72 ± 0.10	149	22.6
FS					
Hexaflumuron (F_{14})	0.043	0.031-0.064	1.32 ± 0.16	–	–
Flufenoxuron (F_{14})	0.007	0.005-0.011	1.10 ± 0.14	–	–

[a]Assays as Table 2. [b]LC_{50} resistance ratio (RR1) compared with FS strain (Tables 1, 3). [c]LC_{50} resistance ratio (RR2) compared with unselected CH strain (Table 1).

diflubenzuron has previously been reported for the cotton leafworm, *Spodoptera littoralis* (Ishaaya and Degheele 1988).

Table 4. Effect of topically-applied synergists, piperonyl butoxide (PB) and S, S, S-tributyl phosphorotrithioate (DEF) on the toxicity of chlorfluazuron and teflubenzuron in a leaf-dip bioassay with second instar larvae of the susceptible (FS) strain of DBM[a,b].

Product (generation)	LC_{50} (ppm ai)	95% FL	Slope ± SE	SR[c]
Chlorfluazuron (F_{18})	0.020	0.015-0.028	1.33 ± 0.15	–
Chlorfluazuron + PB (F_{18})	0.019	0.014-0.026	1.53 ± 0.18	1.0
Chlorfluazuron (F_{20})	0.019	0.010-0.037	1.35 ± 0.17	–
Chlorfluazuron + DEF (F_{20})	0.019	0.010-0.035	1.52 ± 0.20	1.0
Teflubenzuron (F_{18})	0.025	0.018-0.036	1.29 ± 0.16	–
Teflubenzuron + PB (F_{18})	0.022	0.015-0.031	1.42 ± 0.18	1.2
Teflubenzuron (F_{20})	0.024	0.019-0.030	1.32 ± 0.11	–
Teflubenzuron + DEF (F_{20})	0.024	0.019-0.029	1.47 ± 0.12	1.0

[a]Assays as Table 2. [b]PB (10,000 ppm ai) and DEF (20,000 ppm ai) were applied (0.2 µl, uncalibrated volume) to the dorsal thoracic segments 1 hour before larvae were placed on pesticide-treated leaves. Control mortality for synergists alone was 0-4%. [c]LC_{50} synergism ratio (SR).

Table 5. Effect of topically-applied synergists, piperonyl butoxide (PB) and S,S,S-tributyl phosphorotrithioate (DEF) on the toxicity of chlorfluazuron and teflubenzuron in a leaf-dip bioassay with second instar larvae of ATA and NOM-selected subpopulations of DBM (CH strain)[a, b].

Strain (product)	LC_{50} (ppm ai)	95% FL	Slope ± SE	RR[c]	SR[d]
ATA-selected					
Chlorfluazuron (F16)	1.09	0.688-1.93	0.83 ± 0.10	74.7	–
Chlorfluazuron + PB (F16)	0.256	0.159-0.446	0.84 ± 0.12	17.5	4.3
Chlorfluazuron (F20)	0.807	0.573-1.20	0.76 ± 0.07	55.3	–
Chlorfluazuron + DEF (F20)	0.273	0.195-0.399	0.82 ± 0.08	18.7	2.9
NOM-selected					
Teflubenzuron (F16)	2.73	1.65-4.54	0.72 ± 0.10	149	–
Teflubenzuron + PB (F16)	0.165	0.074-0.291	0.73 ± 0.11	9.0	16.5
Teflubenzuron (F20)	1.21	0.793-1.97	0.68 ± 0.07	66.1	–
Teflubenzuron + DEF (F20)	0.355	0.238-0.505	0.74 ± 0.07	19.4	3.4

[a]Assays as Table 2. [b]Synergists applied as Table 4. Control mortality for synergists alone was 4-8%. [c]LC_{50} resistance ratio (RR) compared with FS strain (Tables 1). [d]LC_{50} synergism ratio (SR).

The possibility that the synergists may have acted nonspecifically by enhancing cuticular uptake of pesticides (see Anderson et al. 1986b) was considered unlikely as care was taken to minimize direct interactions between synergist and the pesticide. Also, neither synergist affected the toxicity of acylureas against the FS strain.

In contrast to the present study, Perng et al. (1988) found that while PB almost completely restored the susceptibility of a teflubenzuron-selected field strain of DBM from Taiwan to

teflubenzuron, DEF had no effect. This suggested microsomal oxidation was the major or only resistance mechanism in this instance. However, Cheng (1990) refers to only partial synergism of teflubenzuron or chlorfluazuron by PB in various field populations of DBM in Taiwan. Kobayashi et al. (1990) reported that PB did not synergize the activity of chlorfluazuron in acylurea-resistant field populations from Thailand.

Enhanced microsomal monooxygenase activity (to model substrates) has been directly related to teflubenzuron resistance in field strains of DBM from Taiwan (Lin et al. 1989). Microsomal oxidation has also been suggested to be a major resistance mechanism for pyrethroids in DBM (Liu et al. 1984; Chen and Sun 1986) but cross-resistance was not detected between pyrethroid-resistant and acylurea-resistant strains of DBM (Perng and Sun 1987; Perng et al. 1988; Fahmy and Miyata 1990; Sun 1990). Such observations could be accounted for by different molecular forms of microsomal monooxygenases with varying substrate specificity (Perng et al. 1988; Sun 1988, 1990; Lin et al. 1989).

Multiple forms of the microsomal cytochrome P_{450} are known in insects (Yu and Terriere 1979), while a single point mutation has been shown to be sufficient to completely reverse the substrate specificity of an equivalent mammalian enzyme (Lindbez and Negishi 1989).

The results of the present study and other work on DBM described above clearly indicate that cross-resistance patterns and mechanisms of resistance to acylureas may vary markedly between populations of DBM, some strains remaining susceptible to one group of acylureas while becoming highly resistant to another. However, perhaps more importantly for resistance management in DBM (Cheng 1988, Sun 1990), there is little or no evidence of cross-resistance between acylureas and other insecticides.

Resistance management programs for acylureas are of particular importance, as these compounds (together with *B. thuringiensis*) are especially well suited for use in IPM systems alongside natural enemies. Many other groups of pesticides are less appropriate for such applications although there is, for example, evidence to suggest a relatively selective action for abamectin (Zhang and Sanderson 1990).

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Development and Reversion of Chlorfluazuron Resistance in Diamondback Moth

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Abstract

Selection of two strains (TL and BK) of diamondback moth, *Plutella xylostella* (L.) with chlorfluazuron has produced high levels of resistance in comparatively short time (9 and 10 generations). The resistance ratio values reached 276- and 216-fold, for TL and BK strains, respectively. The BK strain developed about 80-fold resistance level after only three selection generations with chlorfluazuron, suggesting that the resistance gene(s) has , in large measure, been kept in the population. On release from chlorfluazuron selection for two generations, both strains showed no reversion in susceptibility level. Cross-resistance studies revealed very low or no correlation between chlorfluazuron resistance and all insecticides tested in both strains, except teflubenzuron. The two strains showed a slight increase in teflubenzuron resistance level as indicated by resistance ratio values. Heritability (h^2) values for the TL strain was significantly higher than that for the BK strain (0.27 for the TLS strain and 0.064 for the BK strain).

Introduction

With intensive use of chemical insecticides in controlling insect pests, insecticide resistance among target pests has become a great challenge to applied entomologists, because of the widening circle of cross and multiple resistance among insect pests, and the enormous costs of developing new insecticides (Metcalf 1989). The diamondback moth (DBM), *Plutella xylostella* (L.) Lepidoptera:Yponomeutidae), is a cosmopolitan species of considerable importance as a pest of cruciferous crops, especially in Southeast Asia.

The problem of DBM control has become critical, since the insect quickly develops resistance to almost all conventional insecticides soon after the introduction of new compounds (Miyata et al. 1986). In Taiwan, DBM has developed very high levels of resistance to all major groups of conventional insecticides (resistance ratio 100-10000), but still shows susceptibility to the chitin synthesis inhibitors diflubenzuron and chlorfluazuron (Perng and Sun 1987).

Benzoylphenylureas (BPUs) are a new and promising group of insecticides that act primarily on the inhibition of chitin synthesis when ingested, although contact action has been reported. They show larvicidal as well as ovicidal activity against a number of lepidopterous, coleopterous and dipterous insect species (Perng and Sun 1987; Grosscurt 1987).

Teflubenzuron and chlorfluazuron show excellent action in inhibiting the development of DBM (Kohyama 1986; Lim and Khoo 1986), as well as inhibition of chitin synthesis in many other lepidopterous pests (Gijswijt 1979). Recently cases of development of DBM resistance to BPUs has been reported in Thailand (Sinchaisri et al. 1989; Kohyama et al. 1989), in Taiwan (Sun et al. 1990), and in Japan (Tanaka 1990). Generally in countries of Southeast Asia and in Japan, field populations of DBM become resistant within 2-5 years (Cheng 1988).

Field strains of DBM resistant to BPUs are always resistant to many other insecticides, and it is not easy to understand the mechanism of BPU resistance using these field-resistant strains. In this study we tried to obtain a chlorfluazuron-resistant strain by laboratory selection.

A good understanding of the mechanism of development and reversion of resistance to this group of insecticides, as well as cross-resistance patterns, may offer valuable information in order to overcome this problem.

Materials and Methods

Insects

Two strains of DBM used in this study were imported from Thailand: Tup Luang strain (TLS) and Bang Khae strain (BKS). They had been reared in the laboratory free from insecticidal pressure for more than 2 years.

The insects were reared at 24°C under 16L:8D condition. The rearing method was slightly modified from that of Yamada and Koshihara (1978).

About 50 pairs of DBM pupae were introduced to a screened cage (26 × 36 × 29.5 cm). After adult emergence, previously germinated radish seedlings (*Raphanus sativus* cv. Osaka 4) were introduced for oviposition. Hatched larvae were left to feed on the same seedlings until the second instar, then they were transferred to a paper padded plastic box (23 × 30 × 8 cm) and fed on fresh cabbage leaves (*Brassica oleracea* var. *capitata* sub. var. *chuseikanran*) until pupation.

Insecticides

Insecticides used in this study were chlorfluazuron, fenvalerate, phenthoate, thiodicarb, and *Bacillus thuringiensis* Berliner formulation. All insecticide preparations were done by mixing with spreading agent at the desired concentration.

Testing technique and selection

At first the two strains were tested for susceptibility to all the insecticides mentioned, then each strain was divided into two parts: one was reared free from any insecticidal pressure and the other was subjected to selection pressure with chlorfluazuron.

The leaf-dipping technique adopted by Japan Plant Protection Association (JPPA 1988) was used with a slight change. Third instar DBM larvae were used. Cabbage leaves (5 × 5 cm) were dipped for 10 sec in an insecticide solution containing 200 ppm spreading agent (Linoh, Nihon Noyaku co.), and air-dried at 24°C for 2 hours. Leaves were then introduced into a paper padded plastic cup (200 ml) and 10 larvae were placed on each leaf. For control tests cabbage leaves were dipped in distilled water containing spreading agent only. For each insecticide concentration as well as control, at least four replicates were used.

Mortalities were recorded 1 week after treatment in the case of chlorfluazuron and teflubenzuron, 4 days in the case of *B. thuringiensis* and 3 days for all the other insecticides. Larvae which did not respond to pencil tip prodding were considered dead.

For selection experiments the same technique was adopted using at least 1000 larvae for each application. Selection was started using low concentrations, then the concentrations were gradually increased in order to avoid elimination of minor resistance genes if any. The progenies of the selection-surviving insects were divided into two groups, one was used for the next selection and the other for toxicity tests. Both selected and nonselected strains were tested for susceptibility to chlorfluazuron at almost every generation.

Statistics and heritability estimates

The data of susceptibility tests were used to compute dosage mortality regressions according to Probit analysis (Finney 1971).

Heritability (h^2) can be estimated as the ratio of response to selection, to the selection differential, and the slope of the resulting regression line is the heritability (Falconer 1989; Tanaka and Noppun 1989):

h^2 = response to selection/selection differential.

Results and Discussion

Development and reversion of resistance

Selection of both TL and BK strains with chlorfluazuron resulted in high levels of resistance towards this compound in a comparatively short time (9-10 selection generations). After the last selection generation, resistance ratio (RR) values of LC_{50} for TLS and BKS strains were 276 and 216, respectively (Fig. 1 and 2). The RR values expressed here were quite high because they were calculated by dividing the LC_{50} of the selected strain by the LC_{50} of the nonselected strain of the same generation. These nonselected strains were kept without exposure to chlorfluazuron and their LC_{50} values decreased to a very low level compared with the parents. The low values of the LC_{50} of the nonselected strain affected the RR values. When recalculated

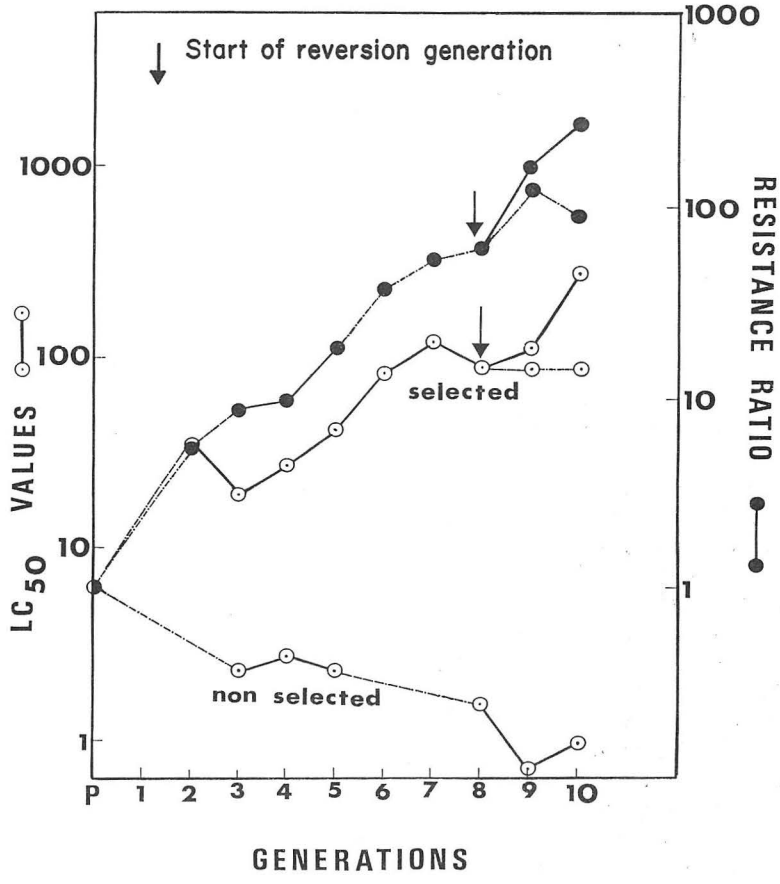

Fig. 1. Development and reversion of chlorfluazuron resistance in TL strain of DBM due to selection pressure.

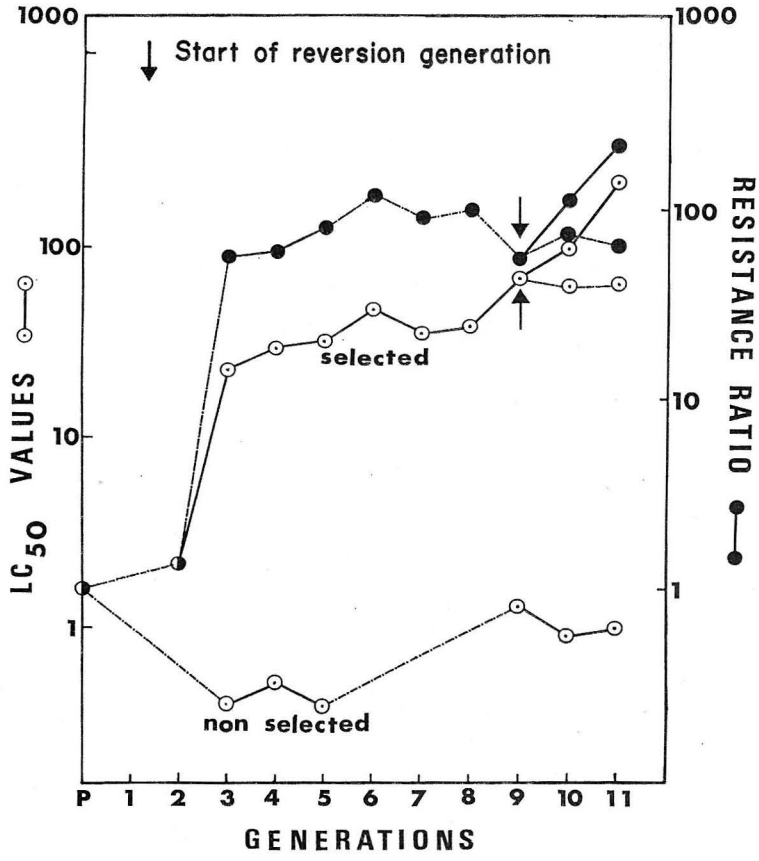

Fig. 2. Development and reversion of chlorfluazuron resistance in BK strain of DBM due to selection pressure.

by dividing by LC_{50} of the parents, the RR values decreased sharply, especially in the case of TL strain. The same phenomenon was observed by Noppun et al. (1987) while selecting DBM with fenvalerate.

High levels of resistance due to selection of DBM were also recorded in many other cases. Noppun et al. (1986), after selecting the Osaka susceptible strain of DBM with phenthoate for eight generations, found this strain exhibited 194-fold of resistance. Higher levels were reported by the same authors, when selecting three strains of DBM with fenvalerate the resistance ratios at LC_{50} level were 55, 580, and 3500 (Noppun et al. 1987).

Sun et al. (1990) observed > 100-fold carbofuran resistance in a susceptible strain of DBM after 10 selection generations. Low level of resistance was also reported by Perng et al. (1988) after 29 selection generations with teflubenzuron. The susceptible strain of DBM has gained only 12-fold resistance, and the rate of development of resistance was slower than expected.

However chlorfluazuron selection for 15 generations produced no resistance in a Taiwanese strain of DBM, as reported by Cheng et al. (1988). The rate of development of chlorfluazuron resistance was rather high in the BK strain, since it had developed about 80-fold resistance level after only three selection generations with chlorfluazuron. This may suggest that the resistance gene(s) has been kept in the population at fairly high frequency. On removal of chlorfluazuron selection pressure, the two strains showed no significant reversion of the level of resistance as indicated by the values of LC_{50}s.

Reduction in pressure was allowed after 8 and 9 selection generations for TL and BK strains, respectively. After two relaxed generations the LC_{50} of the TL and BK strains did not show any remarkable change (Fig. 1 and 2). These results are completely different from those reported by Sinchaisri et al. (1989). The resistance levels of field-collected DBM strains to BPUs decreased sharply a few generations after collection.

This low rate of regression could be due to the prevalence of resistant individuals in the population and their ability to compete with the susceptible ones in terms of reproductive potential, longevity and other biotic factors, as reported by Georghiou (1963). However, Brown (1958) stated that insecticide resistance in insects does not persist once the insecticide pressure is released, and this was observed in both field and laboratory resistant strains of insects. Noppun et al. (1984) reported a decrease of insecticide resistance in DBM after withdrawal of chemicals. A low rate of reversion of insecticide resistance in DBM was also reported by Lee and Lee (1979). The susceptibility levels of DBM to malathion, dichlorvos, diazinon, phenthoate, mevinphos, and endosulfan increased 3- to 7-fold after laboratory rearing for 20 generations. On the other hand, Hama (1983) mentioned that the Miinohara strain of DBM did not show any significant loss of resistance (except to a few insecticides) after laboratory rearing for more than 15 generations. Also DDT and cyclodiene resistance in Danish houseflies has persisted for more than 20 years (Keiding 1977).

Cross-resistance spectrum

Cross-resistance studies on the chlorfluazuron-resistant TL and BK strains to other insecticides revealed very low or no correlation between chlorfluazuron resistance and resistance to the tested insecticides. However the BK chlorfluazuron-resistant strain showed some cross-resistance to teflubenzuron (RR = 9.9) (Table 1).

These results agree with that of Perng et al. (1988) who reported that teflubenzuron-selected strains of DBM did not show apparent cross-resistance either to BPU compounds (except some cross-resistance to PH60-51) or to conventional insecticides. This is also supported by Metcalf (1989), who stated that cross-resistance enables resistant species to survive exposure to chemically related insecticides and generally results from a common detoxification pathway.

However, high levels of cross-resistance in DBM were recorded in many cases. Noppun et al. (1987) recorded high cross-resistance to prothiophos, cyanophos and methomyl in a phenthoate-selected DBM strain. Also laboratory selection studies with DBM provided direct evidence for cross-resistance between pyrethroids and other types of insecticides (Tabashnik et al. 1987).

Table 1. Cross-resistance spectrum to different insecticides in chlorfluazuron-selected and nonselected strains of DBM.

Insecticides	TL Strain				BK Strain			
	Nonselected		Selected[a]		Nonselected		Selected[b]	
	LC_{50}	LC_{95}	RR		LC_{50}	LC_{95}	RR	
			LC_{50}	LC_{95}			LC_{50}	LC_{95}
Phenthoate	21	2380	1.22	.85	20.2	595	2.35	6.9
Fenvalerate	72.6	2630	.81	3.6	114	2740	.65	1.27
Thiodicarb	66	39500	3.15	.90	522	297000	.417	.081
Teflubenzuron	.70	96	3.25	83	.415	91.8	9.93	28
B. thuringiensis	3.38	119	1.04	.52	1.21	125	.762	.65
Chlorfluazuron	.99	10.9	276	628	1.03	18	216	376

[a]$RRLC_{50}$ = LC_{50} of selected strain/LC_{50} of the nonselected. $RRLC_{95}$ = LC_{95} of selected strain/LC_{95} of the nonselected. DBM has been selected for 9 generations. [b]$RRLC_{50}$ = resistance ratio at the LC_{50} level. $RRLC_{95}$ = resistance ratio at the LC_{95} level. DBM has been selected for 10 generations.

Heritability (h^2) estimates

In the selection for resistance to chlorfluazuron, realized heritabilities were estimated as $h^2 = 2.7$ (probability = 0.000021) for the TLS strain, while it was rather low, $h^2 = 0.064$ (probability = 0.03176) for the BKS strain. The basic parameters included for calculation of cumulative selection differentials (ΣS) and cumulative responses (ΣR) among selection regimes for the TLS and BKS strains, respectively, are shown in Tables 2 and 3.

Tanaka and Noppun (1989) used the same method for estimating heritability of phenthoate resistance in two DBM strains. The heritability values for the OKR and OSS strains were $h^2 = 0.42$ (P = 0.00001) and 0.41 (P = 0.00001), respectively. This suggested that phenthoate resistance was moderately heritable and additive genetic variances had been maintained in both strains.

Table 2. Basic parameters used in estimating heritability value of chlorfluazuron selected TLS strain[a].

G	logx	sd	i	S	ΣS	R	ΣR
P	–	1.33	–	–	–	–	–
1	–	–	–	–	–	–	–
2	.921	1.536	.496	.761	.761	–	–
3	.911	1.808	.496	.896	1.657	-.01	-.01
4	1.003	1.021	.644	.657	2.314	.092	.082
5	1.268	.604	.644	.388	2.702	.265	.347
6	1.651	.938	.720	.675	3.377	.383	.730
7	1.720	.685	.880	.603	3.98	.069	.799
8	1.778	1.27	1.271	1.614	5.594	.058	.857
9	2.223	.906	1.159	1.05	6.644	.445	1.302
10	2.437	.851	1.058	.900	7.544	.214	1.516

[a]G = generation. logx = log LC_{50} of the selected strain - logLC_{50} of nonselected strain. sd = Standard deviation of the regression line = 1/slope function. i = Selection intensity (Falconer 1989), S = Selection differential. ΣS = Cumulative selection differential. R = Response to selection. BR = Cumulative response. P = parent.

Table 3. Basic parameters used in estimating heritability values of chlorfluazuron selected BKS strain[a].

G	logx	sd	i	S	ΣS	R	ΣR
P	–	1.068	–	–	–	–	–
1	–	–	–	–	–	–	–
2	.345	1.548	.57	.882	.882	–	–
3	1.745	1.148	.57	.654	1.536	1.4	1.4
4	1.777	.752	.57	.428	1.964	.032	1.432
5	1.905	.530	.798	.423	2.387	.128	1.56
6	1.738	.611	.798	.487	2.874	-.167	1.393
7	1.63	1.565	.798	1.248	4.122	-.108	1.285
8	1.67	1.084	.966	1.047	5.169	.040	1.325
9	1.745	1.295	1.40	1.813	6.982	.075	1.4
10	2.0509	.784	1.058	.830	7.812	.306	1.706
11	2.333	.907	1.058	.959	8.771	.2821	1.988

[a]For explanation of G. log x. sd, i. S, ΣS, R, ΣR and P, please see Table 2 footnotes.

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Biochemical and Physiologial Characteristics of Insecticide Resistance in Diamondback Moth

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Abstract

A pyrethroid-resistant strain of the diamondback moth, *Plutella xylostella* (L.), originally collected in Thailand showed cross-resistance to all the pyrethroids tested. Upon further selection with fenvalerate in the laboratory, the strain exhibited an extremely high LD_{50} value, 41 μg/larva, for fenvalerate. In genetic studies of the resistance, the F_1 progeny showed recessive gene(s), while the B_1 and F_2 progenies had a more complex genetic nature. The in vivo dynamics of fenvalerate applied topically showed: (1) reduced cuticular penetration; (2) increased detoxification; and (3) insensitivity at the site of action being responsible for the resistance mechanism. A large portion of the increased detoxification was due to cytochrome P_{450}-dependent monooxygenases, judging from a remarkable synergism of fenvalerate toxicity by piperonyl butoxide. The resistance was unusually unstable in the absence of selection pressure. The LD_{50} decreased about 50% in every generation. Selection pressure once with fenvalerate or even with malathion, however, increased the LD_{50} restoring the fenvalerate resistance. There was no significant difference in the fitness between the resistant strain and its revertant. These results may imply the presence of an unknown factor(s) necessary to maintain the insecticide resistance in diamondback moth, which cannot be explained by the conventional preadaptation theory.

Introduction

A unique feature of insecticide resistance in the diamondback moth (DBM) (*Plutella xylostella* (L.)) (Lepidoptera:Yponomeutidae) is that the development of resistance can take place quickly. At the same time, the insect can lose resistance fairly quickly if the population is freed from insecticidal pressure. That has been the case with orgnophosphorus and carbamate resistance as well as with *Bacillus thuringiensis* Berliner resistance.

We also found that pyrethroid resistance in a DBM strain originally collected in Thailand was extremely unstable. The results on mechanisms, genetics, and instability of the resistance in the strain are presented below. We believe an understanding of the mechanism of disappearance of resistance is as important as understanding the mechanism of development of resistance. Both may eventually enable us to develop a method to control insecticide resistance.

Cross-Resistance Spectrum

The resistant (R) strain used in the present paper was originally collected in Thailand in 1983, and maintained in our laboratory without exposure to insecticides for 6 months prior to the study. The R/S ratio of LC_{50} indicated that the R strain was cross-resistant to all the

pyrethroids tested and to DDT, suggesting the involvement of KDR factor which is a common mechanism for pyrethroid and DDT resistance (Osborne and Smallcombe 1983).

The R strain further selected with fenvalerate in the laboratory showed a remarkably high LD_{50} for fenvalerate, i.e. 41 μg/larva and the R/S ratio of 8000-fold (Table 1). The strain was resistant to some extent to the other insecticides as well.

Table 1. Resistance factor to several insecticides of the R strain after further selection with fenvalerate in the laboratory.

Compound	LD_{50} (μg/larva)		R/S
	S	R	
Fenvalerate	0.005	41	8200
Malathion	0.19	21	110
Carbaryl	31	>100	>3.2
Vamidothion	>100	>100	–

Source:Maekoshi and Motoyama (1987).

Mechanisms of Fenvalerate Resistance

As the first step of mechanism studies, the in vivo dynamics of topically applied ^{14}C-fenvalerate to the 4th instar larvae was compared between the R and S strains. The treated larvae were placed in the holding vial, and maintained with a small piece of cabbage leaf at 25°C. Volatile metabolites and CO_2 in the expired air were trapped and measured using a liquid scintillation counter. However, no significant radioactivity was detected from either trap at any time period investigated.

The larvae were washed with acetone to determine the external radioactivity, and then homogenized with methanol to determine the internal radioactivity. The radioactivity in the holding vial was fractionated with chloroform and water, and they were regarded as rub-off and excreta, respectively. Three different doses of fenvalerate, equivalent to LD_{50} and LD_{90} of the S strain and LD_{50} of the R strain, were applied.

A difference between the strains is shown in Fig. 1. The R strain showed a slower rate of cuticular penetration and a smaller amount of radioactivity in the internal extract than the S strain. The R strain also showed a higher rate of excretion than the S strain. Similar results were observed regardless of the doses applied.

The cuticular penetration of fenvalerate was further compared between the R and S strains without the influence of excretion factor. Larvae were fixed on a piece of scotch tape after anesthetization and received ^{14}C-fenvalerate topically at four different doses. The radioactivity in the internal extract representing the amount of fenvalerate penetrated was later measured.

The results obtained are shown in Fig. 2. The left side of the graph shows that fenvalerate penetrated at a significantly slower rate in the R strain. The graph on the right of Fig. 2 shows results of a similar experiment except that cuticular wax of both strains was removed by washing the larvae with acetone prior to the application of ^{14}C-fenvalerate. The wax removal almost doubled the penetration rate as can be seen from the different scale of the ordinates, although the R/S difference persisted. The mechanism of the slower penetration therefore lies somewhere beneath the wax layer of the cuticle.

The effect of piperonyl butoxide, a methylenedioxyphenyl compound which inhibits oxidative degradation of insecticides and is used as a synergist, on the cuticular penetration of ^{14}C-fenvalerate was examined. When a mixture of ^{14}C-fenvalerate at 1:3 ratio was applied topically, the rate constant of fenvalerate penetration rather decreased in both strains, although the interstrain difference remained at about the same level. An in vitro measurement of penetration rate using common cutworm skin and a diffusion cell system also confirmed the conclusion (Maekoshi 1988).

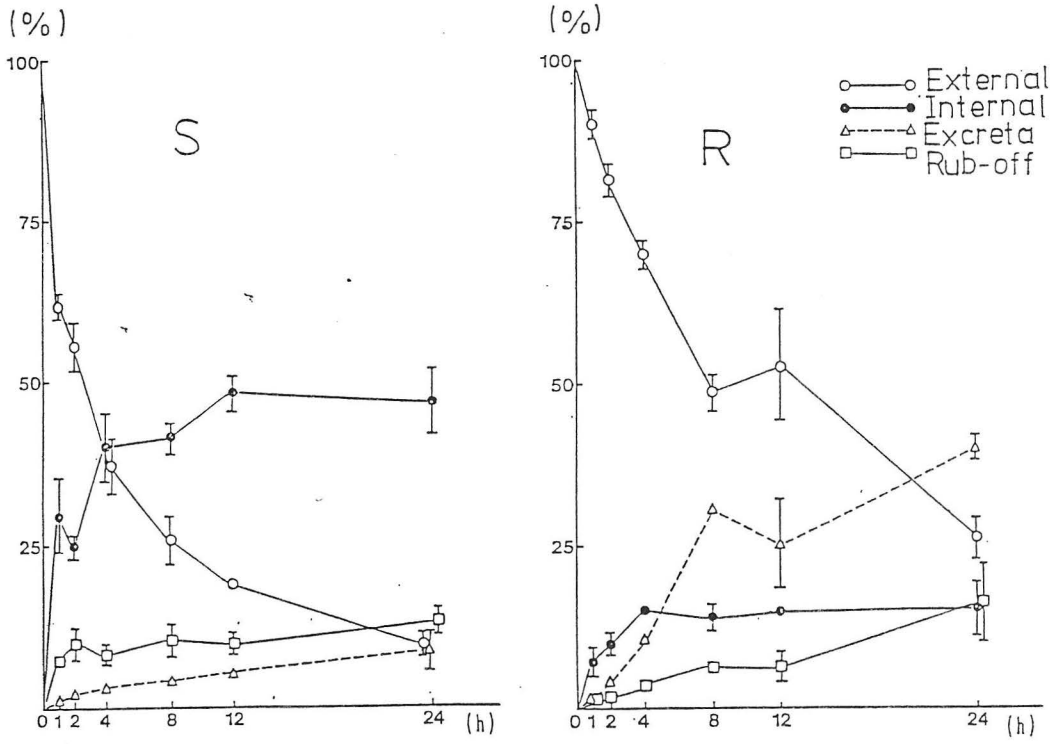

Fig. 1. In vivo dynamics of ^{14}C-fenvalerate (25 μg/larva) applied topically to the S and R larvae. Source: Maekoshi and Motoyama (1987).

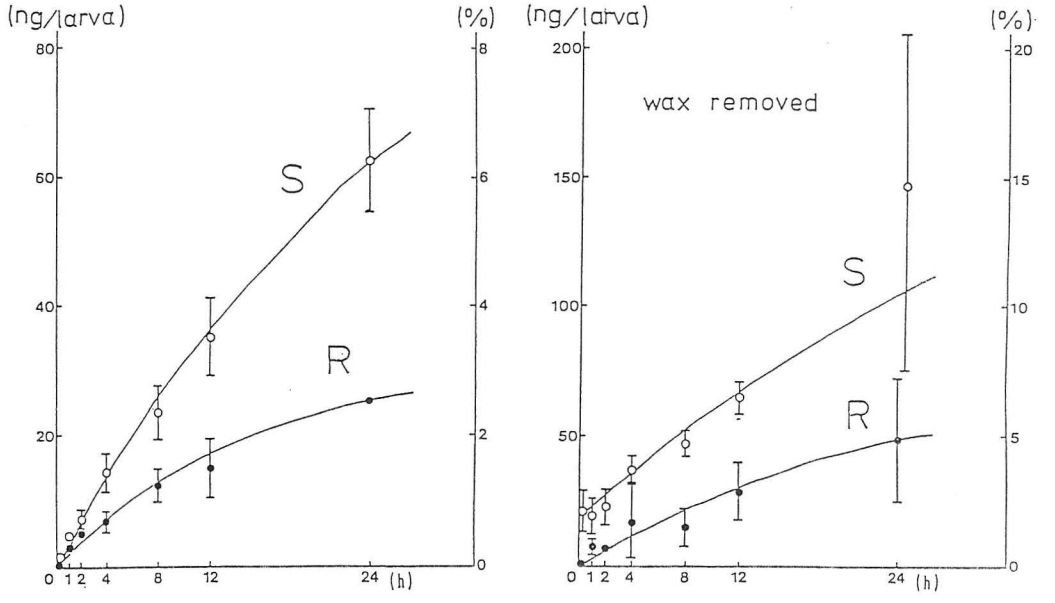

Fig. 2. Cuticular penetration of ^{14}C-fenvalerate (1 μg/larva) applied topically to the S and R larvae. Cuticular wax was removed prior to the application in the right graph. Source: Maekoshi and Motoyama (1987).

The effect of piperonyl butoxide on fenvalerate toxicity to the R strain was examined. Pretreatment of the R larvae with 2.5 μg of piperonyl butoxide resulted in a 150-fold synergism, decreasing the LD_{50} of fenvalerate from 40 to 0.27 μg/larva. The results suggest that increased metabolism of fenvalerate by the cytochrome P_{450}-dependent monooxygenase system is at least in part responsible for the resistance mechanism.

The thin layer radiochromatogram of the internal extract following the application of ^{14}C-fenvalerate demonstrated the presence of several metabolites in addition to fenvalerate itself. The production of these metabolites was compared between the R and S strains. At 1 and 24 hours after the topical application of ^{14}C-fenvalerate, the total radioactivity in the internal extract was significantly less in the R strain than in the S strain as a result of less cuticular penetration. The rate of metabolite formation, however, was higher in the R strain than in the S strain, confirming the increased ability of the R strain to degrade fenvalerate.

In conclusion, the extremely high level of fenvalerate resistance in the DBM strain can be attributed to a multiplying effect of three mechanisms: (1) reduction in cuticular penetration; (2) increase in metabolism of which a large portion is mediated by the cytochrome P_{450} monooxygenase system; and (3) insensitivity at the site of pyrethroid action which is called KDR factor.

Genetics of Resistance

Susceptibility to fenvalerate of F_1, F_2 and B_1 progenies from the S and R strains was determined. The Log Dosage-Probit line of the F_1 progeny derived from either crossing method was located very close to that of the S strain, suggesting the mode of genetics of fenvalerate resistance being essentially recessive (Fig. 3). A slight deviation of the LD-P line toward the right direction might be due to the presence of more than one resistance mechanism with different degrees of dominance.

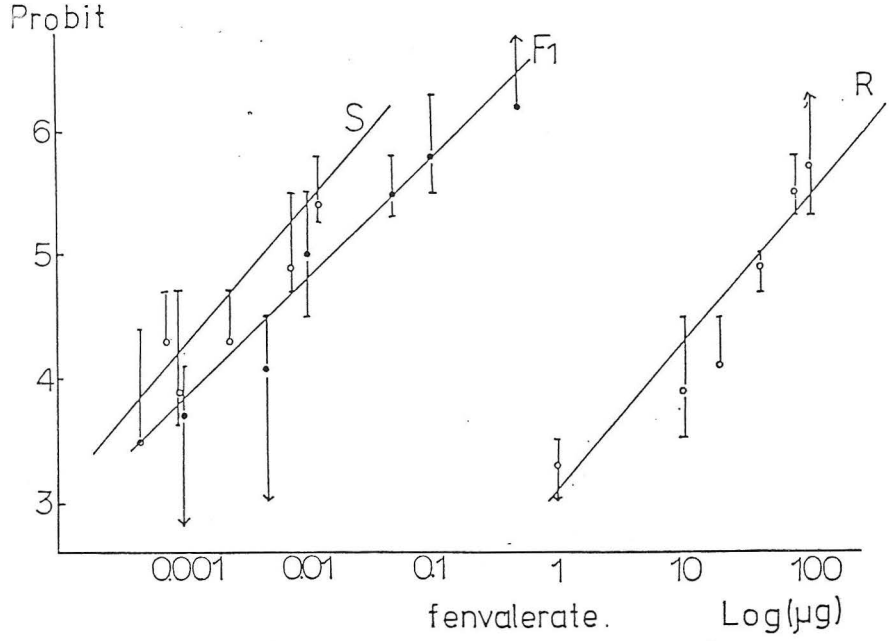

Fig. 3. The LD-P line of the F_1 progeny obtained by crossing S♀ × R♂. Source: Suganuma and Motoyama (1986).

The LD-P lines of the backcross progeny B_1, and F_2 which is not shown here, suggested the genetics of fenvalerate resistance being of a complex nature (Fig. 4). At least it is not monofactorial. The four results may be due to either the effect of three mechanisms involved or unstable nature of fenvalerate resistance in the DBM as will be discussed later.

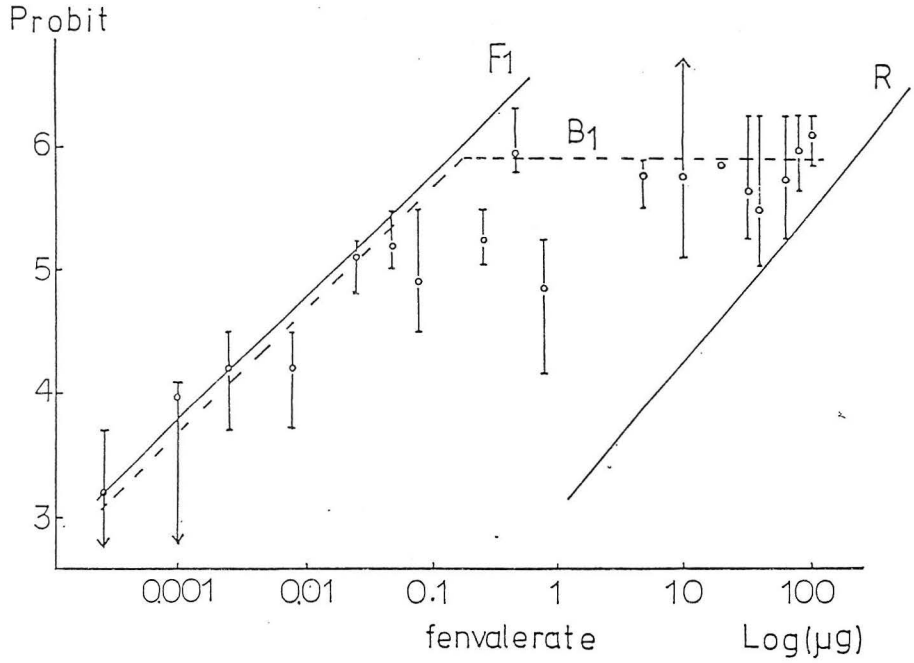

Fig. 4. The LD-P line of the B_1 progeny obtained by crossing F_1 (S♀ × R♂)♂ × R♀. Source: Suganuma and Motoyama (1986).

Instability of Resistance

The R strain has been kept under successive selection with 20 μg of fenvalerate per larva, which stabilized the LD_{50} at about the 40 μg level. Judging from (a) the high LD_{50} value, (b) the sharp slope of the LD-P line, (c) the recessive nature of the genetics, and (d) the stability of the LD_{50}, the R strain appears to be highly homogeneous. However, Fig. 5 shows what happens with the R strain once the selection pressure is removed. As soon as the pressure was removed, the LD_{50} dropped from about 40 μg to 20 μg, 10 μg, 5 μg, and then dropped gradually thereafter.

At the 8th generation after the termination of selection, the strain was divided into two groups: one was kept without selection, and the other was selected again with 20 μg of fenvalerate. The selection immediately increased the LD_{50} to 40 μg, restoring the initial level of resistance, which dropped again without the selection pressure.

At the 11th generation after the termination of selection, a similar attempt was made with 0.1 μg of fenvalerate, a dose insufficient to kill larvae of the strain. The treatment, in contrast, did not restore the resistance at all, indicating that the restoration of resistance observed with 20 μg of fenvalerate was not due to induction, but due to intrinsic selection of resistance mechanisms.

At the 14th generation after the termination of selection, the LD_{50} became as low as 0.2 μg/larva (Fig. 5). When the strain at this generation was selected with 50 μg of malathion, which killed more than 50% of the larvae, the LD_{50} of fenvalerate increased almost three times. On

the other hand, the strain maintained without insecticidal pressure kept losing the resistance spontaneously, eventually reaching the level of the S strain.

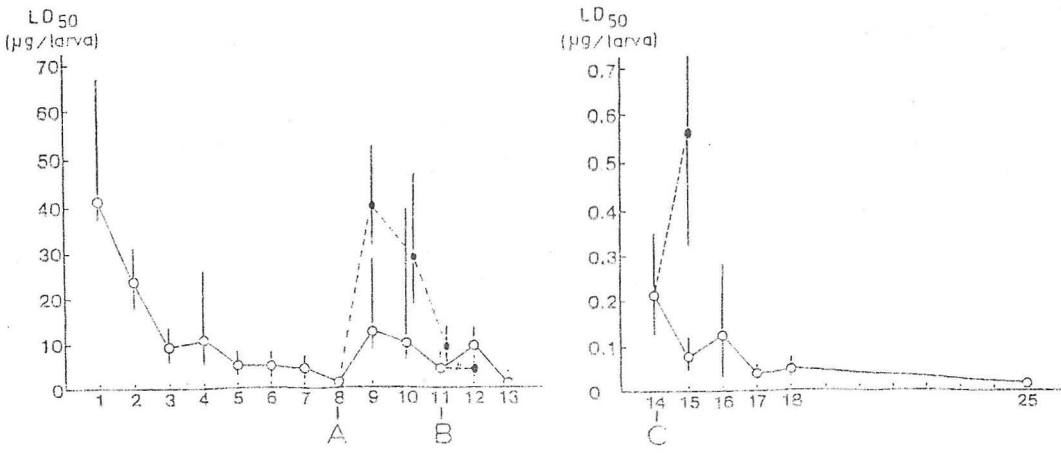

Fig. 5. Change in response to fenvalerate in the R strain following the termination of selection pressure, and the effect of resuming selection with 20 μg (A) or 0.1 μg (B) of fenvalerate or 50 μg (C) of malathion per larva. Source: Maekoshi (1988).

Ecological Fitness

Disappearance of insecticide resistance in some cases has been attributed to a disadvantage in the ecological fitness of resistant strains (Inoue 1980). In other words, under natural conditions where there is no insecticidal pressure, the reproductive ability of a resistant strain would be inferior to that of a susceptible strain. In order to compare various factors determining the reproductive rate, two substrains were produced from the R strain in the present study. One was maintained under successive selection with 20 μg of fenvalerate, and the other was maintained without selection for 33 generations. The latter unselected R strain showed the same level of LD_{50} as the S strain. The two substrains were reared at five different tempeatures: 15, 20, 25, 30, and 35°C.

Table 2 contains a summary of data obtained at 25°C, showing no difference between the selected and unselected R strains in the following characteristics: life span of adult female, length of oviposition period, total number of eggs produced per female or per milligram weight of female, percent pupation, percent adult emergence, and length of egg period, larval period, and pupal period. Similarly, no significant difference in all of these parameters was observed between the substrains at 15, 20 and 30°C (data not shown). At 35°C, females of both substrains could lay eggs but none of them reached pupal stage.

The developmental zero and effective accumulative temperature calculated from the data showed no significant disadvantage for reproduction for the selected R strain as compared to the unselected R strain. In other words, there was no difference in the ecological fitness which can account for the spontaneous loss of fenvalerate resistance in the R strain.

Conclusion

Although the R strain showing 8000-fold resistance to fenvalerate appeared highly homogeneous, a spontaneous loss of resistance still occurred in the absence of insecticidal

Table 2. Biological data of the R strains selected and unselected with fenvalerate.

Characteristics	R strain	
	Selected	Unselected
No. of pairs tested	11	9
Life span of female adult (days)	8.9±3.5	9.9±3.2
Oviposition period (days)	7.4±2.9	7.8±1.9
Fecundity per female	113.6±3.5	115.4±12.1
Fecundity per milligram weight of female	21.3±5.7	25.9±1.7
Pupation (%)	51.7±12.1	56.7±14.6
Adult emergence (%)	49.8±10.8	55.6±14.8
Egg period (days)	3.2±0.6	3.0±0.6
Larval period (days)	9.9±1.8	9.9±1.2
Pupal period (days)	4.8±0.6	4.6±0.6

Insects were reared at 25°C. Source: Maekoshi (1988).

pressure, despite the fact that there was no disadvantage in the ecological fitness. This is apparently outside the context of conventional preadaptation or postadaptation theory for resistance. The phenomenon may be best explained by assuming a possible existence of an unknown mechanism regulating the expression of resistance genes in DBM. The existence of such a mechanism has already been demonstrated with a resistant clone of the green peach aphid, *Myzus persicae*, which has amplified sequences of DNA encoding carboxylesterase production (Field et al. 1988, 1990). Understanding such a mechanism may eventually enable us to turn off the genetic switch to cancel insecticide resistance which has already developed.

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Insecticide Resistance in Diamondback Moth

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Abstract

More cases of insecticide resistance have been reported in many parts of the world and more information regarding the resistance mechanisms has been obtained through research since 1985. This paper, in addition to giving a brief account of the overall situation, places more emphasis on recent findings of diamondback moth, *Plutella xylostella* (L.), resistance to various categories of insecticides, especially the chitin synthesis inhibitors, with an attempt to integrate these results into recommendations for the resistance management of this insect pest of crucifers. Glutathione *S*-transferase degradation has been found responsible for diamondback moth resistance to parathion and methyl parathion. Very limited cross resistance from these two organophosphorus (OP) compounds to some other OPs is observed. Diamondback moth with high microsomal monooxygenase activity converts more parathion to paraoxon, the potent anticholinesterase metabolite; and paraoxon and methyl paraoxon are conjugated at much reduced rates by glutathione *S*-transferase. Components of microsomal monooxygenases have been observed and studied for the first time in diamondback moth larvae. Both qualitative and quantitative differences have been observed among the susceptible and resistant strains. Implications of these observations in resistance management are elaborated.

Introduction

Significantly more research efforts on diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), resistance to insecticides have been made since the First International Workshop on DBM Management was held in 1985. Occurrence of insecticide resistance in this insect pest has been reported, since then, in several countries outside Southeast Asia, e.g., Japan (Hama 1986), U. S. (Tabashnik et al. 1987), Honduras (Secaira 1988), Australia (Altman 1988); resistance to benzoylphenyl ureas (BPUs) or so-called insect growth regulators (IGRs), which did not exist then, has been detected in some regions (Lin et al. 1989; Vattanatangum 1988); and for the first time, DBM resistance to a microbial insecticide, *Bacillus thuringiensis* Berliner, has been observed in the field in Hawaii (Tabashnik et al. 1990).

In the meantime, rapid assays to detect resistance in some insects, such as *Myzus persicae* (Sulzer), have been developed (Field et al. 1989); insecticide resistance management strategy has been adopted and assessed in the control programs of insect pests on some crops, such as *Heliothis* spp. on cotton (Sawicki 1989; Plapp et al. 1990). Thus it appears appropriate and timely for us to reflect upon the resistance management of DBM, despite the paucity of technical data and the pessimistic view expressed by some that this phenomenon might not be manageable.

This paper will first review our work on DBM resistance to major categories of insecticides and some synergists. Then current insecticide resistance management programs on cotton will be examined briefly to illustrate the influence of economic importance and farming scale on their implementation. Finally, guidelines based on existing information for DBM resistance management will be formulated.

What We Know

Our current understanding of DBM resistance to carbamates, organophosphorus insecticides (OP), pyrethroids, synergists, BPUs, and abamectin is summarized in Table 1.

Among these major groups of insecticides, carbamates are the least effective against even the susceptible DBM larvae (Liu et al. 1982). Common mechanisms of resistance with OPs (acetylcholinesterase insensitivity) (Sun et al. 1986) and pyrethroids (oxidative detoxication) (Liu et al. 1984; Chen and Sun 1986) make them less useful while designing management strategies of insecticide mixing or alternations.

On the other hand, OPs have been and will continue to be an important group of compounds for the control of DBM. Enough variations in chemical structures within OPs have contributed to the wide spectrum of efficacy and varied levels of resistance observed in DBM (Liu et al. 1982). While target insensitivity is found to be one resistance mechanism (Sun et al. 1986), its contribution to overall OP resistance is not fully investigated and appears probably additional to that of the metabolic resistance mechanisms (Kao and Sun 1991).

Glutathione S-transferase conjugation appears to be a major detoxication mechanism for parathion and methyl parathion (Kao and Sun 1991). Considerably higher degradation mediated by this detoxifying enzyme is associated with resistance to these two OPs. Existence of isozymes has been proposed. Involvement of this enzyme in DBM resistance to other OPs or other kinds of insecticides is not clear and remains to be assessed. Carboxylesterase hydrolyzes only malathion to a significant extent. A recent in vitro experiment reveals that microsomal P_{450} monooxygenation is probably not related to DBM resistance to some OPs, e.g., diazinon, azinphosmethyl, prothiophos, and tetrachlorvinphos (Kao and Sun 1991). This is in accordance with previous observations that no significant cross resistance existed between OPs and pyrethroids (Chen and Sun 1986).

Three mechanisms have been proposed for DBM resistance to pyrethroids. Reduced penetration is suggested by Noppun et al. (1989) as a major mechanism for fenvalerate resistance. It is nevertheless difficult to assess the importance of this mechanism as well as that of reduced nerve sensitivity (Liu et al. 1982; Hama et al. 1987) in overall resistance phenomenon. We believe that microsomal P_{450} monooxygenase detoxication constitutes the major factor in pyrethroid resistance of this insect pest (Yao et al. 1988; Hung and Sun 1989). All pyrethroids are significantly synergized by monooxygenase inhibitors, e.g., piperonyl butoxide (PB), MGK 264 and sulfoxide, in terms of killing (Liu et al. 1984; Chen and Sun 1986) as well as knockdown (Chen et al. 1985) effects against DBM larvae and adults.

However, DBM may become resistant rapidly to a combination of pyrethroid and synergist, rendering the latter totally ineffective to enhance the toxicity of the former (Chen and Sun 1986). This resistance to synergist appears unstable and upon the termination of synergist usage, susceptibility may recover to a considerable extent within a short period of time. Limited data indicate that resistance to one synergist will not affect the synergistic effect of the others mentioned above.

Before BPUs were introduced to Taiwan, a survey was made to determine the susceptibility baseline of DBM. One important observation is that there is no cross resistance to this new group of compounds from high levels of existing OP and pyrethroid resistance (Perng and Sun 1987; Perng et al. 1988). This phenomenon, of course, was the basis for the remarkable performance of teflubenzuron and chlorfluazuron when they were first put on the markets. To no one's surprise, DBM mustered up its defense weapons within 1 year and control failures started to appear (Lin et al. 1989). Microsomal P_{450} detoxication again has been shown responsible for the resistance in both laboratory-selected and field-collected strains. Further studies show that different forms of cytochrome P_{450}s are probably involved in the oxidative degradation of BPUs and pyrethroids (Lin et al. 1989; Perng and Sun 1987). This also suggests that DBM possesses a tremendously active and versatile microsomal monooxygenase system, which is ready to cope with whatever compounds having chemical structures vulnerable to oxidation (Chen and Sun 1986; Hung et al. 1990). In this regard, we have observed a definite synergism

Table 1. A summary of insecticide and synergist resistance in DBM.

Insecticide/range	Mechanism	Comment
Carbamates 150-2000xstable	Acetylcholinesterase (AChE) insensitivity Oxidative detoxication	Cross resistance with some OPs Cross resistance to/from PYs
Organophosphorus (OP) 100-50000xunstable in some cases	AChE insensitivity (Ki 20-50x difference) Microsomal oxidation probably not involved Glutathione S-transferase degradation for some OPs Carboxylesterase hydrolysis for malathion	Insufficient to account for the high resistance observed No cross resistance from high levels of PY resistance to some OPs
Pyrethroids (PY) 150-2000xstable	Reduced penetration Nerve insensitivity Microsomal oxidation: major mechanism	Difficult to assess the importance Difficult to assess the importance Strong synergism by piperonyl butoxide (PB), MGK 264, sulfoxide Insignificant cross-resistance to some BPUs Knockdown of both larvae and adults synergized by PB
Piperonyl butoxide (PB) > 12xunstable	Microsomal oxidation	Insignificant cross-resistance to MGK 264 and sulfoxide
MGK264 > 6x unstable	Probably microsomal oxidation	Insignificant cross-resistance to PB and sulfoxide
Benzoylphenyl ureas (BPU) 40-700x unstable (?)	Microsomal oxidation: major mechanism	Unusually high activities of some microsomal oxidations Forms of cytochrome P_{450}s probably different from those involved in PY oxidation
Abamectin 125x (lab.) stability unknown	Microsomal oxidation	

of abamectin by PB in a laboratory-selected resistant strain (resistance ratio of 125) of DBM.

Implications of Studies on Insecticide Resistance and its Management of Other Insect Pests

In a recent paper Devonshire (1989) reviewed the work on insecticide resistance in *M. persicae* for the past 20 years in Rothamsted Experimental Station. Through a detailed understanding of the biochemistry and molecular genetic bases of the resistance mechanism, sensitive and reliable methods for monitoring resistance in field populations have been developed. In terms of overall advancement, this has been the most sophisticated work on insect resistance to insecticides up to now. One significant aspect underlying all these accomplishments is that increased production of esterase E4 through gene amplification is the sole mechanism responsible for *M. persicae* resistance to carbamates, OPs as well as pyrethroids (Devonshire and Moores 1982). This is in sharp contrast with what we know about DBM. Resistance of DBM to various groups of insecticides is attributable to almost all known mechanisms, metabolic and non-metabolic. This phenomenon undoubtedly will render the development of rapid biochemical assays for resistance monitoring in the field and resistance management program for DBM a much more complicated task.

Resistance research over the past four decades has identified more than a dozen factors that can influence the development of resistance (Georghiou 1983). Therefore, designing a scientifically valid and economically acceptable resistance management program for a particular pest, such as DBM, is a very complex job. Table 2 lists several programs of pyrethroid resistance management of cotton pests that have been implemented (Sawicki 1989; Plapp et al. 1990). They all have been established without detailed and quantitative knowledge of resistance mechanisms, genetics or ecology of the pests. Whether mandatory or voluntary, curative or preventive, these programs consist of simple guidelines, such as pyrethroids are allowed only in certain periods of time, or certain types of insecticides should precede or follow pyrethroids. Nevertheless, they have been considered in general to be quite effective.

However, I would like to point out some special features of these programs and try to put DBM resistance management in perspective. The implementation of these programs obviously depends on the fact that cotton farming is of such a large scale and commercial value that pest control measures for this crop can dictate spray schemes on all other crops. In addition, simple culture system (one crop per year and somewhat uniform farming calendar) and relatively longer growing season make possible synchronization of spray schedules and full manifestation of management results. In contrast, cruciferous vegetables are often grown by small landholders around urban centers and in highlands in Asia and many other regions. These vegetables are usually grown year-round as their growing seasons vary and are relatively short. Many generations of DBM (>20 in Taiwan) occur each year and generation overlapping is common. In this connection, compliance with any management program appears to be a challenge very difficult for vegetable growers to meet.

In view of the current programs on cotton, which have been based on very limited knowledge, consisting of simple guidelines and generally considered effective, there is no reason why we cannot put resistance management of DBM to work. With existing knowledge, guidelines can be proposed without making serious errors in recommendation. As new data become available, it can be further fine-tuned.

What We Can Do

Five years ago in the First Workshop we proposed our first set of recommendations with regard to the management of insecticide resistance (Sun et al. 1986). The following represents an updated version.

Table 2. Current insecticide management programs in cotton.

Acaricide resistance		
Zimbabwe 1975	Curative measure	Voluntary program Country divided into three regions; same two acaricides (from different chemical groups) used in each region for 2 years; change to next pair of compounds for 2 years; and so on Successful for 14 years
Pyrethroid resistance		
Zimbabwe 1978	Preventive measure *Heliothis armigera*	Voluntary program Pyrethroid spray scheme on all crops dictated by pest control measures for cotton Pyrethroids allowed only in mid summer, 9-week period (peak flowering and fruiting in cotton season) Only those pyrethroids with effective acaricide activity are recommended Has worked well, but not sure if due to the beneficial effects of the program or to a historical lack of insecticide resistance in *H. armigera*
Egypt 1978	Preventive measure *Spodoptera littoralis*	Mandatory program Pyrethroids used solely on cotton and only against *S. littoralis* First spray of OP/IGR mixture, followed by a single spray of OPs or carbamates alone or with IGRs Control failure rising in 1986-87; increasing resistance levels detected Spraying becoming unsynchronized; pyrethroids used on other crops
Australia 1983	Curative measure *H. armigera*	Voluntary program applied to all host plants Cotton season divided into three stages, maximum three applications of pyrethroids only in stage 2, a 6-week period, one application with synergist PB Selection of only one out of four or five generations Proper timing of spray, pyrethroid being selective only on older (>4 day) larvae First 3 years working well, pyrethroid resistance frequency returning to average in stage 1 Resistance frequency high at beginning of season of fourth year, many localized pyrethroid failures at stage 2 Fifth year lower resistance frequency at beginning; *H. armigera* occurrence low; fewer pyrethroid applications; control failures rare

1. Start management before resistance is detected.
2. Avoid continuous cultivation of crucifers.
3. Observe the economic threshold while spraying insecticides.
4. Do not mix insecticides. It usually cannot delay the onset of resistance and often accelerates and complicates it.
5. Always rotate different groups of compounds, i.e., OPs, pyrethroids, BPUs and *B. thuringiensis*.
6. Limit the use of pyrethroids and BPUs. If OPs are still useful, do not use pyrethroids. Do not introduce BPUs if OPs and pyrethroids, alternatively, can still control DBM.
7. Use BPUs on crucifers of longer growing period in order to manifest their toxicity fully. Use BPUs early in the season, just once if possible.
8. Synergists, such as PB, may be used with pyrethroids and BPUs. But do not use PB continuously.

Publications and information available after this Workshop have shown that DBM has developed in the field several hundred fold resistance to *B. thuringiensis*, and resistance mechanism identified in DBM is similar to that found in *Plodia interpunctella*, i.e., reduced affinity of midgut brush border membrane to *B. thuringiensis* toxin (Gould 1991; Van Rie et al. 1990). Cross resistance within *B. thuringiensis* preparations from the same serotype occurs in DBM and this insect could develop resistance to mixtures of *B. thuringiensis* toxins (Tabashnik et al. 1991). Therefore, it appears that resistance to *B. thuringiensis* will occur despite all our wishful thinking, and immediate measures should be taken to manage DBM resistance to this microbial insecticide.

Acknowledgement

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Insecticide Resistance in Diamondback Moth in Florida

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Abstract

Topical application and leaf residue bioassays were conducted to determine the toxicity of selected insecticides to an apparently fenvalerate-resistant population of diamondback moth, *Plutella xylostella* (L.), from central Florida. A field study was conducted to evaluate selected insecticides for the control of diamondback moth and cabbage looper, *Trichoplusia ni* (Hübner), in cabbage, *Brassica oleracea* L. (*capitata* group). Laboratory strains of diamondback moth established from populations from selected treatment plots of the field study were evaluated for susceptibility to fenvalerate. The laboratory studies indicated that the diamondback moth population was resistant to fenvalerate and methomyl, but susceptible to chlorpyrifos, acephate, endosulfan, and thiodicarb. The field study indicated that chlorpyrifos, endosulfan, mevinphos, and *Bacillus thuringiensis* var. *kurstaki* were considerably more effective at suppressing diamondback moth than cypermethrin, permethrin, methomyl, and thiodicarb. Cypermethrin, permethrin, thiodicarb, and endosulfan were the most effective in controlling *T. ni*. Mixtures of two insecticides improved efficacy apparently due to one component controlling *T. ni* and not diamondback moth and the other component controlling diamondback moth and not *T. ni*. Examination of selected populations from the field study indicated that resistance to fenvalerate increased within one crop season with weekly use of cypermethrin and thiodicarb, but not with chlorpyrifos.

Introduction

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), a worldwide pest of cruciferous crops (Talekar and Griggs 1986), was not difficult to control in Florida until about the mid 1980s. Growers began complaining of failing to control DBM in crucifers in transplant (seedling) production facilities and in the field with insecticides. Since about 1985, DBM has replaced the cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae), as the major target of insect control activities in cruciferous crops in Florida (G. L. Leibee, personal observation). The principal insecticides of choice in Florida were fenvalerate and permethrin which until this time (1985), provided excellent control of the cabbage caterpillar complex, which typically consisted of DBM, *T. ni*, imported cabbageworm, *Artogeia rapae* (L.) (Lepidoptera: Pieridae), and the cabbage webworm, *Hellula rogatalis* Hulst. (Lepidoptera: Pyralidae). Fenvalerate failed to provide adequate caterpillar control in cabbage in the spring of 1985 at the Central Florida Research and Education Center (CFREC) in Sanford, Florida (Leibee 1986a). Fenvalerate controlled DBM in the spring of 1986 at the same location, indicating an apparently susceptible population was present (Leibee 1986b). From the winter of 1986-87 to the present, pyrethroid insecticides in general provided essentially no control of DBM at the CFREC (G. L. Leibee, unpublished data). Magaro and Edelson (1990) reported that failures to control DBM in south Texas were first reported by cabbage producers in the spring of 1987. Personal communication with entomologists during the late 1980s indicated that control of DBM

had become a problem in other states bordering the Gulf of Mexico and generally up the eastern seaboard of the United States. Since resistance to fenvalerate in DBM had been reported in Taiwan (Liu et al. 1982), Japan (Hama 1987), and in other areas of Southeast Asia (Cheng 1988), it was obvious that a similar problem was developing in the eastern United States.

The studies reported herein were conducted to document and characterize the nature of insecticide resistance in DBM in central Florida. Laboratory studies were conducted to determine the toxicity of selected insecticides to an apparently fenvalerate-resistant population of DBM and to determine the ability of selected insecticides to increase resistance levels in the field. Field studies were conducted to evaluate selected insecticides for the control of DBM and *T. ni*.

Materials and Methods

DBM colonies: A population of DBM was allowed to build up to a moderate level in a 0.81-ha field of collards, *Brassica oleracea* L. (*Acephala* group), at the CFREC-Sanford. Prior to starting the spray program, a colony (strain A) was started from 139 pupae reared from larvae taken from the collard field from 22 April to 7 May 1987. A spray program was initiated on 13 May where fenvalerate was applied at 0.224 kg AI/ha in 467 l of water on a weekly basis for 5 weeks. One day after the first application, it was observed that the DBM larvae were much more difficult to kill than the *T. ni* and *A. rapae* larvae that were also present in the collard field, indicating that the DBM population was already somewhat resistant to the fenvalerate. At the end of the 5-week spray program, it was apparent that fenvalerate was ineffective in controlling this population of DBM because the collard plants were completely skeletonized, and upon inspection several hours after spraying, there were no obvious effects of the spray on the larvae present. Another colony (strain B) was started from several hundred adults collected with a sweep net on 15 June, 5 days after the last spray application. Adults were used to start this second colony because the larvae that were present appeared diseased. These two colonies were maintained on rape, *Brassica napus* L., seedlings at 25°C and a 15-hour photoperiod and without exposure to insecticides until needed for the bioassays.

Insecticides: Technical grade materials used in the topical application bioassays were fenvalerate (I. E. DuPont deNemours & Co., Wilmington, Delaware), chlorpyrifos (Dow Chemical Co., Midland, Michigan), and methamidophos (Chevron Chemical Co., San Francisco, California).

Formulated materials used in both the leaf residue bioassays and the field trials were chlorpyrifos (50% wettable powder [WP], Dow Chemical Co.), methomyl (215 g/l soluble liquid [L], I. E. duPont deNemours & Co.), thiodicarb (383 g/l flowable liquid [F], Rhone-Poulenc), and endosulfan (359 g/l EC, FMC). Formulated materials used in the field trials only were cypermethrin (359 g/l EC, ICI Americas), permethrin (383 g/l EC, FMC), mevinphos (479 g/l EC, I. E. duPont deNemours & Co.), Dipel 2X (*Bacillus thuringiensis* var. *kurstaki*, 32.0 BIU/kg wettable powder [WP], Abbott), and Javelin (*B. thuringiensis* var. *kurstaki*, 16 billion *Spodoptera* Units/l, Sandoz). Additional formulated materials used in the leaf residue bioassays, but not in the field, were acephate (75% soluble powder (SP), Chevron Chemical Co.) and fenvalerate (287 g/l EC, I. E. duPont deNemours & Co.).

Bioassays: Bioassays were conducted in March and April of 1989 when strain A was in generation 29 and 30 and strain B was in generation 27 and 28. Topical application and feeding studies were utilized to conduct the bioassays. For topical application, a 0.5 μl droplet of acetone containing the appropriate concentration of technical insecticide was applied with an Arnold type micro-applicator (Burkard Manufacturing Co. Ltd., England) to the middle dorsum of an anesthetized early 4th instar larva (3-4 mg). Generally, each dosage was replicated three times with 10 larvae/replicate. The number of dead and moribund larvae was recorded after 24 and 48 hours. A larva was considered moribund if it could not move one body length after prodding.

Larvae were collected from the colonies, sorted to size, placed in the glass petri dishes lined with filter paper and covered. Larvae were provided with pieces of rape seedlings until anesthesia and dosing. All anesthesia, dosing, and maintenance of the larvae were conducted with the larvae in the petri dish. Prior to anesthesia, the larvae were dislodged from the rape seedlings with a camel-hair brush and the plant material removed to facilitate positioning of the larvae. The larvae were anesthetized with carbon dioxide for 8-10 min in a clear polystyrene plastic box with lid. The anesthetized larvae were removed from the box, dosed, and provided with three rape seedlings per petri dish.

Leaf residue bioassays were conducted by placing early 4th instar larvae (3-4 mg) in glass petri dishes containing pieces of rape seedlings coated with dried residues of formulated insecticides and recording the number of dead or moribund larvae after 24 and 48 hours. The rape seedlings were dipped as whole plants for 5 sec in serial dilutions of the formulated insecticide and allowed to dry. Enough plants were dipped with each dose to provide extra for replenishing the food supply, if necessary, during the bioassay. Each petri dish contained three seedlings (generally two cotyledon leaves and one true leaf completely expanded) excised well into the dipped part of the stem. The petri dish bottoms were lined with filter paper. The range of doses for each insecticide included concentrations representing the low and high recommended field rates in 467 l water/ha and concentrations above and below these.

Field study: 'Golden Acre Yellows Resistant' cabbage was transplanted from seedbeds on 20 and 21 April 1989 into Myakka fine sand at the CFRE-Sanford, Florida. Plots consisted of four 15.2 m rows with a 0.76 m row-spacing and about a 0.28 m plant spacing. Four rows were left unplanted between each plot to provide a separation of 3.8 m. Plots were arranged in four blocks and the blocks were separated by 7.6 m alleyways. All the treatments, except for endosulfan, were assigned to the plots in a randomized complete block design with four replicates. The endosulfan treatments were an afterthought and were assigned to plots at the same end of each block originally not to be used because of high soil moisture resulting from poor drainage. Standard cultural practices for the area were used for fertilization and weed control. Sprays were applied with a tractor-mounted, compressed-air sprayer. Three hollow-cone nozzles (D2-25) were used per row; one overhead and one drop on each side. The delivery rate of spray was 467 l/ha with a boom pressure of about 3163 g/cm^2 (45 psi) and a speed of 3.2 km/hour. Application dates were 11, 16, 23 May and 1, 8, 16 June 1989. Insecticides and rates (expressed as kilograms of active ingredient [AI] per hectare unless stated otherwise) used in treatments consisted of chlorpyrifos at 1.12, cypermethrin at 0.067, Javelin at 1.17 l, methomyl at 1.01, permethrin at 0.22, thiodicarb at 0.84 and 1.12, mevinphos at 1.12, and endosulfan at 1.12. Treatments consisting of a mixture of two insecticides and rates listed above were cypermethrin with chlorpyrifos, cypermethrin with Javelin, chlorpyrifos with Javelin, and thiodicarb with mevinphos. An additional treatment of chlorpyrifos mixed with Dipel 2X at 0.56 kg/ha was included. An untreated check was included. The wetting agent X-77 (Chevron Chemical Co.) was used in all treatments at the rate of 0.62 ml/l of spray.

Plants were sampled on 22 and 31 May, and 7 and 14 June 1989 to determine larval numbers. Four randomly selected plants per plot (two from the middle of each center row) were sampled by carefully cutting the stem below the portion of the plant containing the bud or head and the next four youngest leaves (wrapper leaves in the head stage). The stem was cut below all the foliage if only four leaves were present. The four plants were placed in a plastic bag and transported back to the processing area. Each sample of four plants was placed into a Berlese funnel and subjected to heat for 24 hours. When heads were present, the infested head leaves were pulled away from the head and the uninfested portion of the head was cut out to reduce the amount of plant material that went into the funnel. Larvae were collected into 70% ethyl alcohol. All species except DBM were categorized according to size (small, medium, and large). DBM was categorized according to instar by head capsule size. Ten mature plants were rated for damage on 16 June 1989 using a scale of 1-6 similar to that of Greene et al. (1969). Percent marketability for normal market conditions (no head damage) was based on the proportion of

plants having a damage rating of 3.0 or less. Percent marketability for exceptional market conditions (slight amount of head damage) was based on the proportion of plants having a damage rating of 4.0 or less. Damage ratings were taken from only one plot of the endosulfan treatments due to severe stunting in the other three replicates resulting from excessive soil moisture.

Insecticide resistance development study: Colonies of DBM were established from the untreated check, chlorpyrifos, cypermethrin, and thiodicarb plots of the field trial. Larvae and pupae were collected from each replicate of a treatment and combined to provide at least 100 individuals to start each colony. Collections were made from the untreated check plots on 22 June, the cypermethrin plots on 23 June, and the chlorpyrifos and thiodicarb plots on 26 June 1989. The colonies were maintained on rape seedlings into the F_2 generation until there were enough individuals to determine the toxicity of fenvalerate to each colony using the leaf residue procedure described above.

Statistical analysis: Concentration-mortality response data were subjected to probit analysis (Finney 1971) where applicable. All concentration-mortality response data were corrected for control mortality (Abbot 1925) unless indicated otherwise. Data from the field trial were subjected to analysis of variance and means were separated by Duncan's (1955) Multiple Range Test ($P = 0.05$ level). Damage ratings were not transformed. Percentage data were transformed using arcsine. Larval count data were transformed using log $(X + 1.5)$ for DBM and square root $(X + 1.5)$ for *T. ni*. Actual means are presented in tables.

Results and Discussion

Bioassays: There was a significant difference in toxicity of fenvalerate to DBM strains A and B using topical application based on nonoverlap of the 95% fiducial limits (Table 1). The LD_{50} values of 0.96 and 36.75 μg/larva for strains A and B, respectively, indicated that strain B was about 38 times more resistant to fenvalerate than strain A and that both strains were comparable to the super-resistant strains described by Hama (1987). A very stable kind of resistance is indicated in that strains A and B have retained this high level of fenvalerate resistance after 29 and 27 generations, respectively, without selection. Highly stable fenvalerate resistance in DBM has been indicated in Japan (Hama 1988) and Taiwan (Chen and Sun 1986). There were no significant differences in the toxicity of chlorpyrifos and methamidophos to strains A and B based on overlap of the 95% fiducial limits (Table 1). But, the LD_{50} values indicated that chlorpyrifos was about 2.7 times more toxic to strain A as it was to strain B.

All the leaf residue concentration-response data were subjected to probit analysis, but only the data for fenvalerate, acephate, and thiodicarb fit the probit model well enough to present the LD_{50} and slope values (Table 2). There were no significant differences in the toxicity of fenvalerate, acephate, and thiodicarb to strains A and B using the leaf residue bioassay based on overlap of the 95% fiducial limits. But, the LD_{50} values indicated that fenvalerate was about twice as toxic to strain A than to strain B. Even though the leaf residue concentration-mortality response data for methomyl, chlorpyrifos, and endosulfan did not fit the probit model, the data for concentrations representing 0.5, 1.0, and 2.0 times the maximum recommended field rate were considered valuable when presented with similar data for fenvalerate, acephate, and thiodicarb (Table 3). Results indicated very little difference between strains A and B in response to the insecticides. Data suggest that thiodicarb, acephate, chlorpyrifos, and endosulfan at the maximum recommended field rate would be effective in controlling this resistant strain of DBM where fenvalerate and methomyl would not.

The great differences in the relative toxicity of fenvalerate to strains A and B indicated by the two different bioassay methods might be due to differences in the routes of entry of the toxicant inherent in the two bioassay methods. Topical application relies on contact toxicity only where leaf residue relies on ingestion of the toxicant and probably some toxicity from contact

Table 1. Toxicity of fenvalerate (48 hours), chlorpyrifos (24 hours), and methamidophos (24 hours) to two strains (A and B) of DBM using topical application.

Insecticide and strain (generation)	n	Slope ± SE	LD_{50} (ppm)	95% FL
Fenvalerate				
A (F_{29})	177	0.44 + 0.09	1916.98	283.20 – 9152.03
B (F_{27})	180	2.84 + 0.69	73490.94	20515.52 – 158411.64
Chlorpyrifos				
A (F_{30})	180	2.01 + 0.35	5234.39	2665.07 – 8615.22
B (F_{28})	180	1.24 + 0.24	14149.43	8244.15 – 26645.65
Methamidophos				
A (F_{30})	180	1.02 + 0.17	6748.73	2842.90 – 14459.00
B (F_{28})	180	0.91 + 0.21	6801.80	1663.70 – 22307.00[a]

[a]90% fiducial limits.

Table 2. Toxicity of fenvalerate (48 hours), acephate (24 hours), and thiodicarb (48 hours) to two strains (A and B) of DBM using a leaf residue bioassay.

Insecticide and strain (generation)	n	Slope ± SE	LD_{50} (g AI/l)	95% FL
Fenvalerate				
A (F_{29})	180	0.02 + 0.01	2.43	1.41-4.93
B (F_{27})	180	0.02 + 0.01	4.76	3.37-10.36
Acephate				
A (F_{31})	210	1.45 + 0.32	0.28	0.06-0.54
B (F_{29})	210	1.42 + 0.26	0.50	0.19-0.84
Thiodicarb				
A (F_{31})	177	1.15 + 0.29	0.63	0.10-1.18
B (F_{29})	180	1.63 + 0.31	0.74	0.26-1.23

Table 3. Concentration-mortality (48 hours) response of two strains of DBM exposed to leaf residues of fenvalerate, methomyl, thiodicarb, acephate, chlorpyrifos, and endosulfan representing 0.5, 1.0 and 2.0 times the recommended field rates.

Insecticide	Mortality (%) (SE)			
	Strain	0.5X	1.0X[a]	2.0X
Fenvalerate	A	11 (8)	26 (12)	54 (9)
	B	27 (8)	36 (22)	66 (18)
Methomyl	A	65 (7)	72 (8)	77 (12)
	B	52 (10)	53 (12)	93 (7)
Thiodicarb	A	41 (9)	90 (10)	76 (9)
	B	71 (7)	93 (3)	82 (10)
Acephate	A	92 (4)	96 (4)	100 (0)
	B	100 (0)	87 (8)	100 (0)
Chlorpyrifos	A	72 (16)	82 (12)	78 (7)
	B	65 (0)	88 (7)	84 (10)
Endosulfan	A	86 (4)	100 (0)	89 (6)
	B	82 (4)	93 (4)	96 (4)

[a]1.0X for fenvalerate, methomyl, thiodicarb, acephate, chlorpyrifos, and endosulfan is 0.48, 2.16, 1.92, 2.40, 2.40 and 2.40 g AI/l, respectively.

with the leaf residue. The difference in toxicity to fenvalerate between strains A and B with topical application could indicate slower cuticular penetration in strain B. Noppun et al. (1989) showed reduced cuticular penetration of fenvalerate in resistant strains of DBM. Ingestion of fenvalerate with the leaf material might remove differences in cuticular penetration as a factor in toxicity. Also, the similarity between strains A and B indicated by the leaf residue bioassay might be due to loss of resistance in strain B over time due to lack of selection with fenvalerate to maintain the original levels of resistance.

Field study: DBM and *T. ni* were the predominant species present throughout the experiment. Numbers of DBM were different ($P < 0.05$) among treatments on the last three sample dates (Table 4). There were few differences ($P < 0.05$) in the numbers of *T. ni* among treatments on the first sample date and no differences ($P > 0.05$) on the last three sample dates. Numbers of *T. ni* appeared to be very low on each sample date, but a considerable amount of damage in several treatments was characteristic of that produced by *T. ni*. Since *T. ni* larvae can consume 18.4 times more cabbage than DBM larvae (East et al. 1989), the numbers of *T. ni* present over the entire sampling period were considered high enough in several treatments to cause significant damage. When the numbers of *T. ni* were combined over all sample dates, there were more and greater differences ($P < 0.05$) among treatments (Table 4) which corresponded well with amounts of damage present (Table 5).

In the treatments consisting of a single insecticide, chlorpyrifos, mevinphos, endosulfan, and Javelin were considerably more effective at reducing numbers of DBM than cypermethrin, permethrin, methomyl, and thiodicarb, suggesting a higher level of resistance to pyrethroids and carbamates than to organophosphates, endosulfan, and *B. thuringiensis* (Table 4). Except for thiodicarb, the effects of chlorpyrifos, endosulfan, and methomyl on the numbers of DBM in the field study were in agreement with the results of the leaf residue study (Table 3). Cypermethrin, permethrin, thiodicarb, and endosulfan were most effective at reducing numbers of *T. ni*.

Endosulfan was the only insecticide that was effective against both species and produced the most marketable cabbage in the experiment among the treatments consisting of a single insecticide (Table 5). The remaining single insecticide treatments provided essentially no marketable cabbage under normal market conditions.

The insecticide mixtures resulted in a significant ($P < 0.05$) decrease in damage when compared to the damage in the untreated check and that of components of the mixtures, except for cypermethrin + Javelin. Based on the frequency of damage ratings of 3.0 or less, a significant ($P < 0.05$) increase in marketability over the untreated check was indicated in the treatments consisting of the following mixtures listed in order of decreasing marketability: cypermethrin + chlorpyrifos, thiodicarb + mevinphos, and chlorpyrifos + Dipel 2X. Cypermethrin + chlorpyrifos was significantly ($P < 0.05$) better than all other mixtures. Based on the frequency of damage ratings of 4.0 or less, which represents a relatively high level of insect control, all mixtures resulted in a significant increase in marketability over the untreated check with the increases becoming quite high in all but the cypermethrin + Javelin. In general, based on larval numbers, the increase in efficacy from the mixtures appears to be due to one component controlling DBM and not *T. ni* and the other component controlling *T. ni*, but not DBM. The cypermethrin-chlorpyrifos mixture resulted in the greatest reduction in damage probably because the cypermethrin alone provided the best *T. ni* control and the chlorpyrifos alone provided the best DBM control.

Insecticide resistance development study: The toxicity of fenvalerate to DBM strains established from the untreated check, chlorpyrifos, cypermethrin, and thiodicarb plots indicated an increase in resistance to fenvalerate had developed in the cypermethrin and thiodicarb plots relative to the untreated check and chlorpyrifos plots (Table 6). This increase in resistance to fenvalerate occurred over the 5-week spray period during which no more than three generations of DBM could have occurred based on the shortest development time of 14.55 days from egg

Table 4. Effects of selected insecticidal treatments on larval numbers for DBM and *T. ni* on cabbage.

Insecticide and rate (kg AI or l formulation/ha)	No. 2nd-4th instar DBM larvae/4 plants[a]			Total no. *T. ni* larvae/4 plants[a] for all sample dates
	31 May	7 June	14 June	
Untreated check	7.3 ab	5.0 cde	11.0 bcd	6.3 ab
Chlorpyrifos 1.12	0.8 fgh	2.3 e-h	3.3 def	6.0 ab
Cypermethrin 0.067	7.8 a	10.0 a	31.8 a	0.8 de
Javelin 1.17 l	3.5 bcd	3.7 c-f	5.5 cde	4.5 a-d
Cypermethrin 0.067 + Chlorpyrifos 1.12	0.3 gh	1.7 e-h	2.8 ef	1.8 b-e
Cypermethrin 0.067 + Javelin 1.17 l	1.3 e-h	6.7 bcd	14.3 abc	1.8 cde
Chlorpyrifos 1.12 + Dipel 2X 0.56	1.8 d-g	1.7 e-h	3.5 def	5.5 abc
Chlorpyrifos 1.12 + Javelin 1.17 l	0.8 fgh	1.0 fgh	2.0 ef	4.8 a-d
Methomyl 1.01	6.5 ab	2.3 d-g	17.5 abc	3.8 a-e
Permethrin 0.22	6.8 ab	9.3 abc	24.8 ab	0.5 e
Thiodicarb 1.12	2.8 def	13.0 ab	21.8 ab	0.5 e
Thiodicarb 0.84	6.0 abc	7.7 abc	33.0 a	2.0 b-e
Thiodicarb 0.84 + Mevinphos 1.12	1.0 fgh	1.3 fgh	8.5 b-e	1.5 cde
Mevinphos 1.12	3.0 cde	1.0 fgh	6.3 def	6.3 a
Endosulfan 1.12	0.8 fgh	1.0 fgh	3.5 def	2.8 a-e

[a]Means in the same column followed by the same letter are not significantly different ($P = 0.05$; Duncan's 1955 Multiple Range Test).

Table 5. Effects of selected insecticidal treatments for the control of DBM and *T. ni* on damage ratings (DR) and two levels of marketability in cabbage.

Insecticide and rate (kg AI or l formulation/ha)	Damage rating[a]	% marketable[a]	
		(DR≤3)	(DR≤4)
Untreated check	6.0 a	0.0 f	0.0 d
Chlorpyrifos 1.12	5.2 cd	2.5 ef	10.0 cd
Cypermethrin 0.067	6.0 a	0.0 f	0.0 d
Javelin 1.17 l	5.1 d	0.0 f	20.0 bc
Cypermethrin 0.067 + Chlorpyrifos 1.12	3.5 ef	40.0 d	95.0 a
Cypermethrin 0.067 + Javelin 1.17 l	4.9 d	0.0 f	40.0 b
Chlorpyrifos 1.12 + Dipel 2X 0.56	4.0 e	17.5 e	82.5 a
Chlorpyrifos 1.12 + Javelin 1.17 l	3.9 e	12.5 ef	95.0 a
Methomyl 1.01	5.9 ab	0.0 f	0.0 d
Permethrin 0.22	6.0 a	0.0 f	0.0 d
Thiodicarb 1.12	5.7 abc	0.0 f	0.0 d
Thiodicarb 0.84	5.7 abc	0.0 f	0.0 d
Thiodicarb 0.84 + Mevinphos 1.12	3.7 ef	22.5 e	87.5 a
Mevinphos 1.12	5.4 bcd	0.0 f	5.0 cd
Endosulfan 1.12	3.4 -	50.0 -	100.0 -

[a]Means in a column followed by the same letter are not significantly different ($P = 0.05$; Duncan's [1955] multiple range test). '-' indicates unreplicated. See text.

to adult reported for DBM by Sarnthoy et al. (1989). This is in agreement with Chen and Sun (1986) who found that resistance to fenvalerate in DBM could be increased to an extremely high level within four generations in the laboratory with intensive selection with fenvalerate. These results suggest that cypermethrin and thiodicarb were more effective than chlorpyrifos at selecting for resistance to fenvalerate.

Table 6. Mortality (24 hours) of four strains of DBM exposed to fenvalerate leaf residues. The strains were established from mature cabbage field plots that were untreated (UTC) and treated with chlorpyrifos, cypermethrin, and thiodicarb.

Rate of fenvalerate[a]	Mortality (%) (SE)[b]			
	UTC strain	Chlorpyrifos strain	Cypermethrin strain	Thiodicarb strain
0.0X	10.0 (3.3)	2.7 (1.7)	7.6 (1.6)	6.7 (6.7)
0.5X	17.1 (5.4)	13.3 (3.8)	1.7 (1.7)	0.0 (0.0)
1.0X	33.5 (5.6)	20.8 (7.1)	12.1 (2.0)	8.1 (1.8)
2.0X	36.4 (2.0)	21.8 (2.2)	12.1 (2.0)	9.5 (2.4)
4.0X	36.4 (8.4)	32.9 (3.8)	16.9 (4.3)	20.7 (4.0)
8.0X	42.2 (1.1)	34.5 (1.2)	25.7 (3.1)	30.9 (7.9)

[a]1.0X = 0.48 g AI/l. [b]Not corrected for control mortality.

Conclusions: A DBM population in central Florida was found to be highly resistant to fenvalerate. Laboratory studies indicated that the population was also resistant to methomyl, but susceptible to chlorpyrifos, acephate, endosulfan, and thiodicarb. The field study indicated that chlorpyrifos, endosulfan, mevinphos, and *B. thuringiensis* were considerably more effective at suppressing DBM than cypermethrin, permethrin, methomyl, and thiodicarb. Cypermethrin, permethrin, thiodicarb, and endosulfan were the most effective in controlling *T. ni*. Mixtures of two insecticides were shown to improve efficacy apparently due to one component controlling *T. ni* and not DBM and the other component controlling DBM and not *T. ni*. Laboratory examination of populations from the field study indicated that resistance to fenvalerate in DBM increased within one crop season with weekly use of cypermethrin and thiodicarb, but not with chlorpyrifos.

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Insecticide Resistance in Diamondback Moth in Malaysia

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Abstract

The main problems faced by vegetable growers in Malaysia are pests and diseases. Among the major pests of vegetables in Malaysia, diamondback moth, *Plutella xylostella* (L.) is the most serious. The main method of control has been the use of insecticides. However, outbreaks of this pest occur every 2-3 years. New and old insecticides, with regular use, were found to be ineffective in controlling this pest because of the development of insecticide resistance. Studies were initiated to monitor resistance to the major insecticides used by the growers. Field-collected larvae were reared in the laboratory and tested against selected insecticides using the leaf dip method and the spray tower technique. The LC_{50} values were calculated using probit analysis. The resistance ratio (RR) values were calculated by dividing LC_{50} values for resistant DBM strain and LC_{50} values of a known susceptible strain. Diamondback moth populations in the highland and lowland vegetable-growing areas showed resistance to methamidophos, cypermethrin, teflubenzuron, chlorfluazuron and diflubenzuron. Resistance was also recorded to *Bacillus thuringiensis* var. *kurstaki* in one population of this pest in Cameron Highlands. No resistance was recorded to avermectin and *Bacillus thuringiensis* var. *aizawai*.

Introduction

The diamondback moth (DBM), *Plutella xylostella* L. (Lepidoptera: Yponomeutidae), is the most important pest of crucifers in Malaysia. Since it was recorded in 1925 (Ho 1965) the main method of control has been the use of insecticides. The first comprehensive study on detection of resistance to insecticides was conducted by Sudderuddin and Kok (1978). High levels of resistance were detected to malathion, DDT, chlorpyrifos-methyl, lindane and dichlorvos. Insecticide resistance in DBM was found to be lower towards cartap, methomyl, methamidophos, carbaryl and resmethrin.

In the 1980s, studies on DBM were mainly focused on biological control (Lim 1986; Chua and Ooi 1986; Lim et al. 1986) and integrated pest management (IPM) (Syed et al. 1990; Lim 1986). However, several new insecticides were introduced to control DBM, due to the frequent outbreaks of this pest and the failure of the other control methods. In Malaysia, common insecticides used for DBM control in the last decade were a wide range of synthetic pyrethroids, methamidophos, and various formulations of *Bacillus thuringiensis* Berliner. In the mid 1980s several formulations of insect growth regulators (IGRs) were used by the vegetable growers in the highland and lowland areas.

In 1988, serious outbreaks of DBM were recorded in the Cameron Highlands and in the lowland areas of Malaysia. We suspected resistance development to the IGRs which had been used extensively since 1986. To confirm this, insecticide resistance studies were initiated. Furthermore, it was felt that resistance monitoring in DBM would provide useful information to the existing IPM program in Malaysia.

Materials and Methods

Sites

Three locations were selected in the Cameron Highlands: Tringkap (CTT), Kea Farm (CKF) and Sungai Palas (CSP) and two locations in the lowlands: Jalan Kebun (LJK) and Karak Highway (LKH) around Kuala Lumpur. The DBM populations in the highlands and in Jalan Kebun were collected from cabbage plots while in Karak Highway from Chinese mustard. The growers were interviewed to obtain information on types of insecticides used and frequency of application (Table 1).

Insects

During 1988-89, about 500-1000 DBM larvae were collected from each field. The larvae were reared in the laboratory in the Cameron Highlands on 40-45-day-old potted cabbage (cv. KY Cross) plants in screen cages (42 cm × 43 cm × 55 cm), at 18-28°C with a photoperiod of 12L:12D. With the exception of the susceptible strain (SS), larvae used in bioassays were F_1, F_2 or F_3 offspring of field-collected individuals. The SS colony was obtained from Taiwan (Sun. C. N . National Chung Hsing University, Taichung), and was maintained without insecticide treatments for more than 20 generations before it was bioassayed.

Chemicals

Eight formulated insecticides were used: three IGRs viz., teflubenzuron (Nomolt 15% SC; Hoechst (M) Co. Ltd.) chlorfluazuron (Atabron 1.12% EC; ICI (M) Co. Ltd.), diflubenzuron (Dimilin 25% WP; Serba Kimia (M) Co. Ltd.); one macrocyclic lactone viz., avermectin (Avermectin 1.8% EC; Hoechst (M) Co. Ltd.) two *Bacillus thuringiensis* var.: *aizawai* (Florbac FC, 8500 IU/mg; Zeenex (M) Co. Ltd.) and *kurstaki* (Dipel WP, 16 000 IU/mg: Oriental Agricultural Products (M) Co. Ltd.); one organophosphorus, methamidophos (Tamaron 600 48.4% LC; Bayer (M) Co.) and one pyrethroid, cypermethrin (Ripcord 505 5.66% EC; Thiram Kimia (M) Co. Ltd.).

Bioassays

The leaf dip method (Tabashnik and Cushing 1987) was used for the IGRs and *B. thuringiensis*, while the spray tower (Potter 1952) technique was used for methamidophos and cypermethrin. For the leaf dip method, leaf disks (5 cm diam) were cut from fully expanded cabbage leaves grown from seeds in the greenhouse. Disks were dipped for 10 sec in distilled water solutions of formulated insecticide with wetter/spreader (Extravon; Ciba-Geigy (M) Co. Ltd; Rate 0.5 ml/l) and air-dried at 23°C for 2 hours. Each disk was then placed inside disposable plastic cups (6.5 cm × 6.0 cm × 4.5 cm). Ten to 15 DBM larvae (4-day-old, early 3rd instar) were placed on the disk (one replicate) and allowed to feed for 48 hours at 23°C:15°C (D:N). Larval mortality was checked daily.

For the spray tower method, 10-15 larvae were anesthetized with CO_2 for 1 min in a petri dish and placed on the spray tower holding stage. The anesthetized larvae were sprayed with 3 ml of the insecticide and placed on untreated cabbage disk in plastic cups as described above.

At least five insecticide concentrations plus a control (distilled water with spreader/sticker) was included in each test. Each test was replicated at least four times. The overall control mortality was <0.1% (range 0-1.5% per test). For the IGRs, *B. thuringiensis* and avermectin analysis was conducted on mortality after 5 days, while for cypermethrin and methamidophos analysis on mortality was done after 3 days.

Analysis

Data were analyzed by the probit procedure (SAS Institute 1985), using the 'C =' option for control mortality. Resistance ratios (RR) were calculated by dividing the LC_{50} of each field population by the LC_{50} of the SS population.

Results and Discussion

Results of the probit analysis are shown in Tables 2-3. The RRs for CSP, LJK and LKH populations were 4.96, 16.99 and 7.22 respectively. The population in Jalan Kebun showed resistance to cypermethrin even though only permethrin and deltamethrin were used in this area. This is probably due to development of cross-resistance. Cross-resistance in pyrethroids is well documented (Sudderuddin and Kok 1978; Liu et al. 1981; Tabashnik 1986; Tabashnik et al. 1987). The lower RR in the Cameron Highlands population is probably due to the shift in pattern in insecticide usage among the growers. The growers in the Cameron Highlands began using mainly teflubenzuron and chlorfluazuron in 1986, 1987 and early 1988. Other compounds were used only 3-4 times in each crop cycle (Table 1).

The RRs for methamidophos in CSP, LJK and LKH populations were 5.3, 123.2, 307.7 respectively. The lowland populations showed high levels of resistance to methamidophos, which was ineffective against DBM. The lower RR value in the highlands was expected due to the rare usage of methamidophos during this period.

The two common IGRs used in most of the crucifer-growing areas were chlorfluazuron and teflubenzuron. These insecticides were considered 'miracle' compounds in 1986 and 1987, when production of cabbage almost doubled in most of the cultivated areas. However in early 1988 these compounds were ineffective and serious outbreaks of DBM became apparent. Resistance was found in all the populations tested both in the highlands and the lowlands. However, the RR was significantly higher in the lowlands, even though these compounds were also extensively used in the highlands. The reason for this is unknown. One of the factors that may contribute to this phenomenon could be the generation time of DBM in the highlands is longer

Table 1. Background information on DBM field sites (1986-87).

Site (elevation)	Crop (duration)	Insecticides	
		Type	no.spray/crop cycle
Highlands			
Tringkap (1220 m)	Cabbage	Pyrethroids	3-4
Kea Farm (1372 m)	(90 days)	IGRs (Teflubenzuron,	4-6
Sungai Palas (1830 m)		Chlorfluazuron)	
		Bacillus thuringiensis	0-3
		Methamidophos	3-4
Lowlands			
Jalan Kebun (50 m)	Cabbage	Pyrethroids	8-10
	(90 days)	(Permethrin, Deltamethrin)	
		IGRs (Teflubenzuron,	3-4
		Diflubenzuron)	
		Bacillus thuringiensis	8-10
		Methamidophos	8-10
Karak Highway (76 m)	Chinese	Pyrethroids	6-8
	mustard	IGRs (Teflubenzuron)	6-8
	(35 days)	*Bacillus thuringiensis*	6-8
		Methamidophos	6-8

(28 days) than in the lowlands (14 days). Among other factors, high temperatures are also conducive to insecticide resistance development in DBM (Yamada and Koshihara 1978).

Development of resistance to diflubenzuron was not surprising since this compound was used in the vegetable-growing areas for almost 12 years. In the early 1980s diflubenzuron was found to be effective to control DBM in the lowlands, but was not effective in the highlands areas. This is probably due to the development of resistance in the highlands much earlier than in the lowlands.

No resistance was recorded to avermectin (Table 2) in populations tested in the Cameron Highlands. This compound is not registered for use in Malaysia. However, avermectin was found to be very effective in controlling DBM (Syed and Hee 1989).

Bacillus thuringiensis has been used by the growers since 1965 for the control of DBM. However, it was not popular among the vegetable growers due to the slowness in its activity in killing DBM. The serotype generally used in the 1960s was *B. thuringiensis*, then *B.*

Table 2. Susceptibility of DBM to various insecticides.

Population	n	LC_{50} µg (AI)/ml	RR[a]
Cypermethrin			
SS	240	9.43	1
CSP	280	46.81	4.96
LJK	280	160.25	16.99
LKH	280	68.12	7.22
Methamidophos			
SS	280	21.95	1
CSP	240	116.35	5.30
LJK	320	2703.50	123.16
LKH	240	6754.85	307.73
Chlorfluazuron			
SS	240	0.057	1
CSP	280	0.56	9.82
CTK	280	0.54	9.47
CKF	240	1.62	28.42
LJK	200	58.46	1025.61
LKH	280	16.63	291.75
Teflubenzuron			
SS	280	0.46	1
CSP	480	6.73	14.63
CTK	240	6.45	14.02
CKF	240	8.22	17.86
LJK	480	1440.00	3130.43
LKH	200	1361.14	2959.00
Diflubenzuron			
SS	200	60.98	1
CTK	280	2171.82	35.61
LJK	480	1907.00	31.27
Avermectin			
SS	240	0.013	1
CKF	240	0.024	1.84
CSP	280	0.042	3.23

[a]RR = LC_{50} Field/LC_{50} SS.

thuringiensis var. *kurstaki* was introduced in the 1980s. Recently, *B. thuringiensis* var. *aizawai* was registered for the control of DBM in Malaysia. This serotype gave effective control of DBM (Syed and Hee 1989). The serotype *kurstaki* showed high levels of resistance in Kea Farm populations in the Cameron Highlands (Table 3). This formulation was found to be ineffective in several areas in the Cameron Highlands, and today *B. thuringiensis* var. *azawai* is mainly used. No resistance was found in the DBM population tested with *B. thuringiensis* var. *aizawai*.

Current levels of resistance in Malaysia are high especially for the IGRs, and these chemicals are not recommended for use in the IPM program. Therefore usage of IGRs should be reduced drastically with increased emphasis on biological control. This is in line with the IPM approach adopted in Malaysia in 1988, where biological control was given priority. Therefore constant monitoring for insecticide resistance should be undertaken to provide information in formulating new strategies in the management of DBM.

Table 3. Susceptibility of DBM larvae to strains of *Bacillus thuringiensis*.

Population	*B. thuringiensis* var. *kurstaki*			*B. thuringiensis* var. *aizawai*		
	n	LC_{50} μg (AI)/ml	RR[a]	n	LC_{50} μg (AI)/ml	RR[a]
SS	320	0.04	1	320	0.21	1
CKF	280	4.50	112.50	240	0.70	3.33

[a]RR = LC_{50} Field/LC_{50} SS.

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Diamondback Moth in South Texas: A Technique for Resistance Monitoring in the Field

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Abstract

Laboratory bioassays were conducted to determine the response of larvae of a susceptible strain of diamondback moth *Plutella xylostella* (L.) from the Rio Grande Valley of south Texas to five classes of insecticides. Test insecticides were permethrin, methamidophos, endosulfan, methomyl and *Bacillus thuringiensis*. Three monitoring techniques were developed and tested as methods of determining the extent of insecticide resistance in the field. The most efficient technique involved a disposable cup assay in which larvae were exposed to discriminating doses of insecticide representing LC_{90} doses for a susceptible strain. Monitoring tests confirmed resistance to permethrin and methamidophos in one field population. Tests with a second field population showed methomyl was the most effective insecticide available. Field data substantiated the results of the monitoring test. The applicability of resistance monitoring methodology developed with *Heliothis* in U.S. cotton to problems of resistance management in diamondback moth are presented.

Introduction

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), is a cosmopolitan pest of cruciferous crops and a key pest of cabbage in the Rio Grande Valley of south Texas where there is extensive production of these crops from fall through spring each year. Resistance to insecticides has been slower to appear in DBM of the United States mainland compared to elsewhere in the world. First reports of control difficulties in the Rio Grande Valley led to confirmation of resistance in DBM populations in 1987 and 1988 (Magaro and Edelson 1990). In this paper we will briefly describe the development of resistance and our responses to the situation. The aim of the research was to confirm the presence of resistance and to provide growers with a rapid and easy-to-use method for determining which class of insecticides would provide control in a field situation where the presence of resistance was suspected. The development of techniques to do this was influenced by parallel studies performed in 1986 and 1987 for determining the presence of resistance to pyrethroid insecticides in the tobacco budworm (TBW), *Heliothis virescens* (F.), in Texas and other southern states (Leonard et al. 1987; Luttrell et al. 1987; Plapp et al. 1987).

Methods

When resistance appeared in the Rio Grande Valley, the first problem was to develop a rapid method to monitor for it. Three different techniques were developed and tested. These included a leaf dip assay, a treated vial assay and finally, a disposable cup assay. The latter has proven to be the most useful under field conditions.

The initial problem with the assays was to decide between the use of a dose-mortality response and the use of a discriminating dose assay. The former can tell the investigator the resistance of the average insect in the population; the latter tells what proportion of the population is resistant. Earlier work with the TBW had led to the development and wide use of a discriminating dose assay which provides information on the proportion of the population resistant to an insecticide. Data over several years of tests with this technique and approximately 60,000 field-collected TBW males have been reported (Plapp et al. 1990). The advantages of using a discriminating dose technique have been described by Roush and Miller (1986) and include greatly simplified assays and much more efficient statistical treatment of the data. As we will show below, the use of this assay is very useful in determining the types of insecticides that may be useful for DBM control in situations where resistance is present.

The initial assay method used by Magaro and Edelson (1990) was a leaf dip assay. First, tests were done at a dose designed to be equivalent to field rates of permethrin, esfenvalerate, methomyl, carbaryl, methamidophos, methyl parathion, and endosulfan. These insecticides are representative of four different chemical classes of insecticides and three different modes of action, e.g., sodium channel agents, cholinesterase inhibitors and a GABA agent. The results showed resistance was present to all compounds and highest to the cholinesterase-inhibiting organophosphorus and carbamate insecticides.

The next step was to determine dose-mortality lines for susceptible DBM with the same insecticides. This was done with permethrin, endosulfan, methamidophos, methomyl and also *B. thuringiensis*. From this work, the authors were able to determine the LC_{90} doses for each insecticide to a susceptible strain. It is this determination which is critical to developing discriminating dose assays.

Tests were done using the glass vial technique at LC_{90} doses based on extrapolations from the previously described assay. The method worked well, but proved less useful than the method described below.

The technique finally decided on was defined as a Disposable Cup Bioassay. This technique is very close in theory and practice to the leaf dip assay. For this technique discriminating dose concentrations of several insecticides were poured into 29.6 ml plastic cups, swirled around and the excess poured out. DBM larvae were then placed in the cups and response determinations made 4 hours later.

The different techniques were tested on laboratory-reared DBM larvae and the results were found to be comparable with each method. Because of ease of use and lower costs, the disposable cup assay was finally adopted as the technique of choice.

Two series of tests were performed in which DBM larvae were collected from fields where resistance was suspected. Larvae were tested with several potential control agents and the decision as to which insecticide to use was based on the results obtained. In one test endosulfan proved to be the insecticide of choice. In a second test, methomyl was chosen. Assays of the results proved that the method was effective in predicting if a particular insecticide would yield satisfactory control.

Nature of DBM Resistance

An important point to be considered in dealing with insecticide resistance is to determine its cause in a pest population. There are two main mechanisms of resistance in most pest species. These are changes at target sites and increases in ability to detoxify insecticides (metabolic resistance). The latter type is most prevalent in polyphagous insects while the former type is more prevalent in monophagous or oligophagous pests. The DBM is clearly an example of the former type. Evidence that resistance to DDT and pyrethroids is target site has been reported (Liu et al 1982b) and evidence for insensitive acetycholinesterase (target site) resistance to organophosphates has also been reported (Noppun et al. 1987). Resistance associated with each type usually yields populations with 10-100-fold resistance. When higher levels are present,

the cause is usually interaction of both factors. In the DBM, there is evidence in a number of cases of greater levels of resistance. An excellent example is the high level of resistance to pyrethroids in the Ban-chau strain (Liu et al. 1982a).

Available data from the Rio Grande Valley research as well as from many previous studies suggests that target site resistance is present in almost all resistant DBM populations. Metabolic resistance, when present, seems to be an additional factor. Indeed, both types of factors are almost always present in cases of high resistance levels.

An easy way is available to determine which type of resistance is present in a field population. The assay is based on the understanding that high levels of metabolic resistance to insecticides occur only in actively feeding life stages, e.g. large larvae. Target site resistance, on the other hand, will be present in all life stages. Therefore, determination of resistance levels in adults and neonate larvae as well as in larger larvae can be used to determine the type of resistance present.

From work with *Heliothis* we know there are biological costs to insects associated with both metabolic and target site resistance to insecticides. Target site resistance to pyrethroids is clearly associated with decreased reproductive success and decreases in mating competitiveness. Metabolic resistance in the tobacco budworm is associated with decreases in fecundity including increases in generation time and decreases in egg production per female (Campanhola et al. 1991).

Management of Resistance in DBM

As discussed above, an understanding of the mechanisms of resistance is crucial for developing adequate resistance management strategies. In the case of the tobacco budworm, pyrethroid resistance is usually associated with target site changes. Successful management has been obtained by restricting pyrethroid use to one generation per year and using alternate types of insecticides if control is necessary for other generations. It seems likely that the same principle can be applied to insecticide control of the DBM.

Metabolic resistance, if present instead of or in addition to target site resistance, can be managed by using mixtures of insecticides. Examples include use of insecticide:synergist mixtures and also use of insecticides composed of mixed isomers. Examples of the latter include ±isomer organophosphorus compounds such as methamidophos and oxime carbamates such as methomyl. Attempts to utilize either approach in managing resistance have not been successful, providing strong evidence that target site resistance is the major factor present in most resistant DBM populations.

In practice, determining the mechanism of resistance is secondary to determining if resistance of any sort is present. Monitoring techniques as described by Magaro and Edelson (1990) and reviewed here provide a way to resolve this problem. If growers can use these techniques to make appropriate control decisions, pest management can be improved and selection for insecticide resistance may be decreased. Overall, monitoring can play an important role in the successful implementation of IPM programs for the DBM.

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Insecticide Resistance of Diamondback Moth in North America

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Abstract

The extent and geographic distribution of resistance to methomyl, permethrin and methamidophos in 44 populations of diamondback moth, *Plutella xylostella* (L.) from 19 states within the U.S., Mexico, Canada and Belize was determined in 1988. Widespread resistance to all three insecticides was confirmed. Resistance was generally highest in populations originating from southern states but scattered populations with high levels of resistance were also detected in northern states. In most instances where resistance was detected to one insecticide, there was also resistance to the other two. Highest levels of resistance were detected for methomyl. During 1989, diamondback moth was imported into New York on southern cabbage transplants (seedlings). During June, when most transplants arrived in New York, DBM infestations were as high as 12.8 insects per 100 transplants on an individual shipment. Compared to a standard susceptible field population, the diamondback moth which were collected from transplants had moderate to high (> 100-fold in one case) levels of resistance to permethrin and methomyl. In 1990, eleven diamondback moth populations were surveyed for susceptibility to two commercial formulations of *Bacillus thuringiensis*. High levels of resistance (in some cases > 200-fold) were found in populations which originated from Florida.

Introduction

The diamondback moth (DBM) *Plutella xylostella* (L.) (Lepidoptera:Yponomeutidae) is a key pest of cruciferous crops throughout the world. In tropical and subtropical areas, crucifer production has been seriously affected in recent years by DBM which has developed resistance to a wide range of insecticides (Sun et al. 1986). Georghiou (1981) has reported DBM resistance to 36 insecticides in 14 countries. In North America DBM has normally been considered a minor pest in the lepidopteran complex, but in the last 5 years entomologists in several states (including Florida, Georgia, North Carolina, Texas, Wisconsin and New York) have reported economic damage in crucifers as a result of their inability to control DBM. Such control failures may have been influenced by environmental factors (Harcourt 1986; Sastrodihardjo 1986), but when examined on a regional basis, insecticide resistance is the most tractable cause.

Our studies on the extent and development of insecticide resistance in DBM within North America occurred in three phases. In 1988 we surveyed the level of susceptibility of DBM to three commonly used synthetic insecticides (methomyl, permethrin and methamidophos) representing the three major classes of insecticides (pyrethroids, carbamates and organophosphates) used within North America. As a result of information gathered in 1988 which indicated high levels of resistance in New York, we initiated a study in 1989 to determine if resistant DBM populations in New York were the result of importing DBM on plants grown in southern states. The third phase of this study surveyed the level of susceptibility of 11 DBM populations to two commercial formulations of *Bacillus thuringiensis* Berliner.

Insecticide Resistance to Methomyl, Permethrin and Methamidophos

In 1988, cooperators were asked to collect 50-150 DBM larvae and pupae from commercial or research cabbage fields in their respective areas. Collections were made in each area during the peak of DBM activity. Forty-four populations from 19 states within the U.S., Mexico, Canada and Belize (Tables 1-3) were evaluated using a leaf dip bioassay similar to Tabashnik et al. (1987) for susceptibility to permethrin (Ambush 2E), methomyl (Lannate 1.8L), and methamidophos (Monitor 4E). We attempted to test all field-collected populations in the first five generations; populations from Geneva, New York, Pulehu, Hawaii, and College Station, Texas, were laboratory colonies and were tested in later generations. Data were analyzed using the POLO procedure (Russell et al. 1977) to obtain LC values. Resistance ratios (RR), the ratio of the LC_{50} of a given population to that of the standard population, were calculated. The standard population originated from Geneva, New York, in 1988 and was laboratory-reared for 23 generations.

DBM populations exhibited extreme variation in susceptibility to methomyl (Table 1). A colony from Greenville, North Carolina had the highest RR of 780, followed by RRs of 362

Table 1. Susceptibility to methomyl of DBM larval populations.

Region	Population		N	LC_{50}-48h (95% CL) mg AI/ml		Slope ± SE	RR[a]
East	Lochwood, CT	F4	238	0.960	(0.689-1.333)	2.32±0.28	9.06
	Derry, NH	F3	202	3.430	(2.284-5.072)	1.51±0.19	32.4
	Litchfield, NH	F2	220	3.805	(1.798-6.706)	1.21±0.21	35.9
	Fairton, NJ	F4	243	4.042	(2.424-5.699)	1.87+0.34	38.1
	Albion, NY	F5	242	36.28	(15.61-195.4)	0.77±0.15	342
	Davie, NY	F1	241	0.926	(0.529-1.394)	3.09±0.57	8.74
	Geneva, NY	F23	242	0.106	(0.084-0.129)	3.12±0.68	1.00
	Long Island, NY	F4	251	9.419	(7.104-12.51)	2.14±0.33	88.9
	Ransomville, NY	F3	243	9.299	(6.226-14.62)	1.30±0.19	87.7
	Dover, DE	F8	246	0.883	(0.430-1.722)	1.84±0.19	8.33
Midwest	Celeryville, OH	F3	219	1.176	(0.782-1.742)	1.46±0.16	11.1
	Fremont, OH	F2	241	0.428	(0.144-0.984)	1.19±0.15	4.04
	Simcoe, ONT	F2	237	4.500	(3.152-6.150)	1.82±0.25	42.4
	Lake Co., IN	F2	214	3.119	(1.386-6.780)	1.28±0.15	29.4
	Purdue, IN	F2	240	0.389	(0.268-0.563)	1.90±0.26	3.67
	Holtz, MI	F4	235	1.580	(0.841-2.567)	1.90±0.25	14.9
	Stolz, MI	F4	200	2.905	(1.741-4.507)	1.74±0.22	27.4
	Arlington, WI	F3	241	0.510	(0.33[b]-0.755)	2.48±0.45	4.81
	Funks E. S., WI	F3	233	0.287	[b]	0.94+0.44	2.71
	Funks M. S., WI	F3	235	0.217	(0.167-0.276)	3.78±0.80	2.05
	Heldings, WI	F2	237	0.293	(0.131-0.603)	1.84±0.27	2.76
	Poynette, WI	F2	243	3.995	(2.778-5.587)	1.68±0.21	37.7
Pacific	Nakatani, HI	F2	239	3.462	(1.925-5.739)	1.55±0.19	32.7
	Pulehu, HI	F86	206	0.563	(0.383-1.055)	3.07±0.56	5.31
	Mt. Vernon, WA	F2	213	0.284	(0.221-0.408)	3.25±0.72	2.68
	Yakima, WA	F4	208	0.424	(0.308-0.637)	2.49±0.41	4.00
Southwest	Belize, C.A.	F1	239	38.39	[b]	7.65±85.1	362
	Bixby, OK	F2	193	0.358	(0.250-0.545)	2.05±0.33	3.38
	South Donna, TX	F2	235	5.126	(4.098-6.309)	3.49+0.60	48.4
	Tamu, TX	F9	241	0.544	(0.369-0.908)	2.58±0.36	5.13
	Weslaco, TX	F1	232	2.500	(1.417-4.111)	1.87±0.23	23.6
South	Zellwood, FL	F1	248	4.039	(2.379-5.783)	2.13±0.36	38.1
	Tifton, GA	F1	235	17.96	(12.15-31.37)	1.44±0.26	169
	Greenville, NC	F1	240	82.73	(27.2-15680)	1.06±0.26	780
	Painter, VA	F5	235	0.507	(0.333-0.751)	1.43±0.17	4.78

[a]RR is the resistance ratio determined by dividing the LC_{50} for a population by the LC_{50} for the Geneva population. [b]Neither the 95 nor 90% CL could be estimated because $g > 0.5$.

from Belize, and 342 from Albion, New York. When classified by levels of RR, 11% of the populations had an RR > 100, 6% from 50-99, 29% from 25 to 49, 8% from 10 to 24 and 46% < 10.

Resistance ratios for permethrin were lower than for methomyl (Table 2). Resistance ratios for permethrin were highest in a population collected from Albion (80.8), followed by Belize, (78.4) and Tifton, Georgia (75.9). When classified by levels of RR, 17% of the populations had RRs > 50, 8% from 25 to 49, 19% from 10 to 24 and 56% < 10.

The highest RR for methamidophos was 42.3 for the population collected from Belize (Table 3). When classified by levels of RR, none of the populations had an RR > 50, 3% from 25 to 49, 13% from 10 to 24 and 83% < 10.

Table 2. Susceptibility to permethrin of DBM larval populations.

Region	Population		N	LC_{50}-48h (95% CL) mg AI/ml		Slope ± SE	RR[a]
East	Lochwood, CT	F2	248	0.019	(0.010-0.030)	1.24 ± 0.20	0.58
	Dover, DE	F7	198	0.541	(0.331-0.830)	1.66 ± 0.24	16.4
	Derry, NH	F7	243	0.709	(0.441-1.003)	1.73 ± 0.33	21.5
	Litchfield, NH	F2	238	0.452	(0.289-0.699)	1.29 ± 0.15	13.7
	Fairton, NJ	F3	243	0.261	(0.126-0.470)	2.02 ± 0.24	7.91
	Albion, NY	F4	248	2.669	(1.949-3.810)	2.41 ± 0.59	80.8
	Davie, NY	F1	194	0.020	(0.007-0.040)	0.88 ± 0.15	0.61
	Geneva, NY	F23	195	0.033	(0.017-0.060)	1.65 ± 0.35	1.00
	Long Island, NY	F5	241	0.652	(0.520-0.801)	3.70 ± 0.64	19.8
	Ransomville, NY	F2	244	1.614	(0.925-3.359)	1.19 ± 0.20	48.9
Midwest	Celeryville, OH	F2	246	0.940	[c]	1.03 ± 0.39	28.5
	Fremont, OH	F1	205	0.005	(0.000-0.003[c])	0.47 ± 0.12	0.15
	Simcoe, ONT	F5	234	0.546	(0.269-1.045)	1.28 ± 0.17	16.5
	Lake Co., IN	F1	246	2.070	(0.977-7.630)	1.18 ± 0.18	62.7
	Purdue, IN	F2	241	0.002	(0.000-0.006)	0.88 ± 0.23	0.06
	Arlington, WI	F1	156	0.322	(0.147-0.610)	1.59 ± 0.23	9.76
	Funks E. S., WI	F1	241	0.030	(0.018-0.048)	1.61 ± 0.25	0.91
	Funks M. S., WI	F1	240	0.113	(0.060-0.194)	1.40 ± 0.16	3.42
	Heldings, WI	F1	241	0.060	(0.028-0.105)	1.74 ± 0.27	1.82
	Poynette, WI	F1	247	0.315	(0.163-0.497)	2.18 ± 0.31	9.55
Pacific	Santa Cruz, CA	F4	229	0.004	(0.000-0.012)	0.73 ± 0.17	0.12
	Nakatani, HI	F3	245	0.097	(0.012-0.323[b])	2.02 ± 0.26	2.94
	Pulehu, HI	F86	202	0.033	(0.006-0.097)	1.35 ± 0.20	1.00
	Yakima, WA	F2	248	0.013	(0.007-0.020)	1.78 ± 0.47	0.39
Southwest	Belize, C.A.	F2	237	2.586	(1.883-3.998)	2.07 ± 0.41	78.4
	Celaya, MEX	F3	239	0.145	(0.089-0.231)	1.44 ± 0.15	4.39
	Bixby, OK	F3	234	0.024	(0.012-0.042)	1.30 ± 0.20	0.73
	South Donna, TX	F1	239	1.868	(1.337-2.737)	2.55 + 0.41	56.6
	Tamu, TX	F9	247	0.016	(0.007-0.027)	1.42 ± 0.27	0.48
	Weslaco, TX	F1	245	0.495	(0.390-0.603)	3.83 ± 0.73	15.0
South	Homestead, FL	F1	206	0.451	(0.247-0.836)	1.23 ± 0.14	13.7
	Zellwood, FL	F1	238	1.162	(0.603-2.371)	1.96 ± 0.29	35.2
	Tifton, GA	F1	246	2.507	(1.753-4.089)	2.03 ± 0.41	75.9
	Greenville, NC	F2	229	1.654	(1.064-2.930)	1.23 ± 0.20	50.1
	Painter, VA	F5	220	0.157	(0.087-0.274)	1.61 ± 0.18	4.76

[a]RR is the resistance ratio determined by dividing the LC_{50} for a population by the LC_{50} for the Geneva population. [b]90% CL. The 95% CL could not be estimated because g > 0.5. [c]Neither the 95 nor 90% CL could be estimated because g > 0.5.

Table 3. Susceptibility to methomidophos of DBM larval populations.

Region	Population		N	LC_{50}-48h (95% CL) mg AI/ml		Slope ± SE	RR[a]
East	Lochwood, CT	F1	242	0.150	(0.109-0.207)	2.19 ± 0.27	2.68
	Dover, DE	F3	193	0.224	(0.059-0.344)	2.99 ± 0.84	4.00
	Derry, NH	F7	245	0.223	(0.154-0.318)	2.26 ± 0.27	3.98
	Litchfield, NH	F2	170	0.275	(0.150-0.334[b])	4.68 ± 1.99	4.91
	Fairton, NJ	F4	230	0.556	(0.315-0.860[b])	3.86 ± 0.56	9.93
	Albion, NY	F6	239	0.738	(0.575-0.951)	2.49 ± 0.31	13.2
	Davie, NY	F1	248	0.209	(0.151-0.291)	2.29 ± 0.27	3.73
	Geneva, NY	F29	220	0.056	(0.042-0.071)	2.63 ± 0.41	1.00
	Long Island, NY	F5	243	0.468	(0.314-0.711)	2.44 ± 0.29	8.36
	Ransomville, NY	F3	243	0.354	(0.273-0.458)	2.86 ± 0.36	6.32
Midwest	Celeryville, OH	F3	173	0.203	(0.134-0.301)	1.64 ± 0.23	3.63
	Fremont, OH	F2	231	0.046	(0.032-0.060)	2.44 ± 0.43	0.82
	Lake Co., IN	F3	241	0.131	(0.089-0.179)	2.15 ± 0.32	2.34
	Purdue, IN	F2	245	0.102	(0.062-0.152)	2.01 ± 0.27	1.82
	Holtz, MI	F6	186	0.321	(0.193-0.554)	2.07 ± 0.23	5.73
	Mt. Clemens, MI	F1	221	0.049	(0.034-0.064)	2.74 ± 0.51	0.88
	Big Lake, MN	F2	242	0.058	(0.038-0.078)	2.49 ± 0.46	1.04
	Funks E. S., WI	F1	236	0.045	(0.024-0.067)	2.17 + 0.41	0.80
	Poynette, WI	F1	149	0.247	(0.133-0.362)	2.70 ± 0.62	4.41
Pacific	Santa Cruz, CA	F5	205	0.560	(0.025-0.092)	1.79 ± 0.30	10.0
	Nakatani, HI	F2	217	0.920	(0.421-3.085)	1.62 ± 0.22	16.4
	Pulehu, HI	F86	180	0.333	(0.136-0.472)	3.38 ± 0.93	5.95
	Yakima, WA	F1	152	0.028	(0.012-0.040)	2.75 ± 0.81	0.50
Southwest	Celaya, MEX	F5	200	0.657	(0.475-0.918)	3.25 ± 0.44	11.7
	Belize, C.A.	F1	241	2.371	c	1.90 ± 0.29	42.3
	Tamu, TX	F16	243	0.057	(0.033-0.082)	2.59 ± 0.48	1.02
	Weslaco, TX	F1	240	0.199	c	11.9 ± 125	2.13
South	Zellwood, FL	F2	243	0.096	c	2.21 ± 0.29	1.71
	Tifton, GA	F1	240	0.109	(0.075-0.165)	2.78 + 0.37	1.95
	Painter, VA	F5	241	0.129	(0.098-0.166)	2.30 ± 0.30	2.30

[a]RR is the resistance ratio determined by dividing the LC_{50} for a population by the LC_{50} for the Geneva population. [b]The 90% CL could not be estimated because $g > 0.5$. [c]The 90% CL could not be estimated because $g > 0.5$.

DBM Infestations in Transplants

In 1989, cabbage transplants (seedlings) were obtained from growers or brokers in Ontario, Yates, Monroe, Orleans and Genesee counties of New York who received shipments of transplants from Florida, Georgia, and Maryland (these states supply most of the transplants to New York). We also obtained locally grown transplants (Phelps, New York). Over the course of the spring and summer we sampled 28 different shipments for DBM and other insect pests. Sampling began with a shipment of cabbage from Georgia on 25 April and ended with a shipment of locally grown transplants on 29 June. A sample consisted of approximately 1000 transplants.

As soon as it was received, each sample of transplants was inspected visually for DBM larvae. In addition, transplants were inspected for cabbage looper, *Trichoplusia ni* (Hübner), imported cabbageworm, *Artogiea rapae* (L.), and cabbage webworm, *Hellula rogatalis* (Hulst). DBM larvae collected during the first inspection were counted and transferred to rape seedlings, *Brassica napus*, and reared for insecticide assays. The inspected transplants were then placed

in soil in large pots and kept for two additional weeks, at which time a second inspection was performed to detect larvae that were not eclosed at first inspection.

We were able to establish four colonies of DBM from transplants and tested the F_2 generation using a leaf dip bioassay similar to Tabashnik et al. (1987) for susceptibility to permethrin and methomyl. Data were analyzed using the POLO procedure (Russell et al. 1977) to obtain LC values. Resistance ratios (RR), the ratio of the LC_{50} of a given population to that of the standard population, were calculated. The standard population used in this study originated from Painter, Virginia. This was chosen as our standard because it originated from a location which had a susceptible population (Shelton, A.M., unpublished).

Infestations varied by source and date of collection (Table 4). Highest average DBM infestations for the season were from a Georgia source (3.44 insects/100 transplants) and a Maryland source (3.50 insects/100 transplants). Transplants originating in New York had fewer DBM larvae (0.59-1.10/100 transplants) than other states. The highest infestation on an individual shipment was found in a Florida sample which had 12.8 DBM/100 transplants. The seasonal average for all sources except Maryland had fewer than 0.50 other insects (imported cabbageworm, cabbage looper, and cabbage webworm) per 100 transplants collected. In 3 out of 4 cases, when transplants were sampled from the same source over several months, later samples had much higher infestations. By June, when most of transplants arrive in New York, infestations were as high as 8.2 DBM/100 transplants for a Florida company, and 7.3 DBM/100 transplants for a Maryland company.

Based on LC_{50} values, the two Florida populations had RR of 7.3 and 8.3 to permethrin (Table 5), and 33.0 and 111.2 to methomyl (Table 6) in comparison with the standard population. The Georgia population had an RR of 24.1 to permethrin but only 4.6 to methomyl. The Maryland population had an RR of 12.5 and 13.1 to permethrin and methomyl, respectively.

Table 4. Insect infestation rates for cabbage transplants from southern sources, 1989.

Source	No. Plants	No. insects found per location source[a]							
		April		May		June		Season Average	
		DBM	other[b]	DBM	other	DBM	other	DBM	other
Georgia A	2957	0	0	2.70	0.07	*[c]	*	1.32	0.03
Georgia B	1059	1.32	0.09	*	*	*	*	1.32	0.09
Georgia C	1892	4.26	0.22	*	*	2.70	0.40	3.44	0.32
Maryland	8755	*	*	0.23	0.46	7.30	5.60	3.50	2.80
Florda	5702	0	0	0.27	0.05	8.20	0.06	2.50	0.04
New York A	3190	*	*	*	*	1.10	0.16	1.10	0.16
New York B	1039	*	*	*	*	1.06	0.19	1.06	0.19
New York C	512	*	*	*	*	0.59	0.20	0.59	0.20

[a]Values listed are [No. insects/No. plants inspected) × 100]. [b]Other insects included imported cabbageworm, cabbage looper, and cabbage webworm. [c]No transplants intercepted from source during that particular month.

Table 5. Susceptibility of 3rd instar DBM larvae obtained from southern transplants to permethrin, 1989.

Population source	Generation	Slope	LC_{50} (mg AI/ml)	95%FL (LC_{50})	RR[a]
Painter, Virginia (standard)	F2	1.388	0.083	(0.042-0.146)	1.0
Florida Company A	F2	2.027	0.607	(0.381-0.982)	7.3
Florida Company B	F2	1.717	0.687	(0.182-3.230)	8.3
Maryland	F2	1.004	1.041	(0.271-6.230)	12.5
Georgia	F2	8.425	2.002	X[b]	24.1

[a]Resistance ratio is the ratio of the LC_{50} of a given population to that of the standard population. [b]Neither the 95 nor 90% CL could be estimated because $g > 0.5$.

Table 6. Susceptibility of 3rd instar DBM larvae obtained from southern transplants to methomyl, 1989.

Population source	Generation	Slope	LC_{50} (mg AI/ml)	95%FL (LC_{50})	RR[a]
Painter, VA (standard)	F1	0.882	0.256	(0.094-0.605)	1.0
Florida Company A	F2	2.698	8.458	(5.010-14.21)	33.0
Florida Company B	F2	2.114	28.46	(16.32-52.12)	111.2
Maryland	F2	0.901	3.351	[b]	13.1
Georgia	F2	0.093	1.164	[b]	4.6

[a]Resistance ratio is the ratio of the LC_{50} of a given population to that of the standard population. [b]Neither the 95 nor 90% CL could be estimated because $g > 0.5$.

Resistance to *Bacillus thuringiensis*

In 1990, cooperators collected 50-150 DBM larvae and pupae from commercial cabbage fields in their respective areas. Some collections were made in areas where growers had experienced difficulty in controlling DBM with commercial formulations of *Bacillus thuringiensis* var. *kurstaki*, as well as other areas in which we did not have specific information on its effectiveness. Eleven populations from six states and Indonesia (Tables 7-8) were evaluated using a leaf dip bioassay similar to Tabashnik et al. (1987) for susceptibility to two *B. thuringiensis* products (Javelin WG and Dipel 2X) at 72 and 96 hours post-treatment.

Data were analyzed using the POLO procedure (Russell et al. 1977) to obtain LC values. Resistance ratios (RR), the ratio of the LC_{50} of a given population to that of the most susceptible population, were calculated.

DBM populations exhibited extreme variation in susceptibility to *B. thuringiensis* (Tables 7-8). Because we attempted to run all assays in the first generation (previous studies have sometimes shown dramatic declines in susceptibility to some insecticides (Sun et al. 1986 and Shelton, A.M., unpublished data), we were not always able to select the best set of doses to test against a particular population to bracket a 5-95% response. However, high levels of resistance to the two products were seen in some Florida and New York populations. Conservative resistance ratios (based on the standard having an LC_{50} value lower than the lowest test rate) indicate an RR for Javelin of 211 and an RR for Dipel 2X of 214 for the Albion, NY, population which originated from transplants grown in Florida. High RRs were also noted in populations from Florida.

Table 7. Susceptibility of diamondback moth larval populations to Javelin WP.

Population[a]	Slope ± SE	LC50 (95% CL) mg (AI)/l	Resistance[b]
Belle Glades FL	[c]	0.13[d]	–
Sanford FL	0.54 ± 0.08	4.84 (1.88-12.5)	37
Sarasota FL	0.53 ± 0.09	5.48 (1.94-15.5)	42
Zellwood FL	0.66 ± 0.18	11.94 (2.17-65.6)	92
Tifton GA	[c]	0.13[d]	–
Fletcher NC	1.21 ± 0.28	0.21 (0.11-0.42)	2
Albion NY	0.77 ± 0.22	27.54 (5.08-149)	211
Hilton NY	0.88 ± 0.10	0.47 (0.28-0.79)	4
Penasquitos CA	[c]	0.13[d]	–
Rio Grande TX	[c]	0.13[d]	–
Bogor Indonesia	[c]	0.13[d]	–

[a]Sample size for each experiment was 150. Mortality was assessed 96 hours after treatment. [b]Resistance ratio is the LC_{50} divided by the LC_{50} of the most susceptible population. [c]Slope for a logit regression could not be estimated because the lowest concentration caused about 100% mortality. [d]95% CL for the LC_{50} could not be estimated because of poor fit of the probit regression model.

Table 8. Susceptibility of diamondback moth larval populations to Dipel 2X.

Population[a]	Slope ± SE	LC_{50} (95% CL) mg (AI)/l	Resistance Ratio[b]
Belle Glades FL	2.14 ± 2.12	0.12 (0.03-0.42)	1
Sanford FL	0.86 ± 0.08	6.27 (3.72-10.5)	63
Sarasota FL	0.44 ± 0.13	6.68 (1.02-43.5)	67
Zellwood FL	0.66 ± 0.16	4.15 (0.94-18.4)	41
Tifton GA	[c]	0.13[d]	–
Fletcher NC	0.91 ± 0.23	0.23 (0.08-0.64)	2
Albion NY	1.04 ± 0.22	21.4 (7.71-59.6)	214
Hilton NY	0.94 ± 0.10	0.51 (0.32-0.80)	5
Penasquitos CA	[c]	2.00[d]	–
Rio Grande TX	1.92 ± 1.69	0.10 (0.03-0.36)	1
Bogor Indonesia	[c]	0.13[d]	–

[a]Sample size for each experiment was 150. Mortality was assessed 96 hours after treatment. [b]Resistance ratio is the LC_{50} divided by the LC_{50} of the most susceptible population. [c]Slope for a logit regression could not be estimated because the lowest concentration caused about 100% mortality. [d]95% CL for the LC_{50} could not be estimated because of poor fit of the probit regression model.

Conclusions

This study is the first one to examine DBM susceptibility over a broad geographic area and the results indicate high levels of resistance to three major classes of synthetic insecticides, as well as two major *B. thuringiensis* products. Resistance was generally highest in the southern states, but scattered populations with high levels of resistance were also detected in northern states.

Insects with high levels of resistance, as indicated by laboratory assays, originated from locations where control failures occurred. This was especially true with populations from Belize, Florida, Georgia, North Carolina, and parts of New York and Wisconsin, the two major cabbage-growing areas in the northern US. Because of the short growing season in New York and Wisconsin and the absence of DBM overwintering in an adjacent area (Ontario, Canada) (Harcourt 1986), high levels of resistance would not be expected in New York and Wisconsin. Our studies indicate that DBM problems in these northern states can originate from DBM which are brought up on transplants grown in southern states. Thus, management of DBM on a regional basis becomes necessary. While it may be possible to restrict the shipment of DBM on plant material, natural migrations into an area may still occur. Information on the abundance of DBM entering into an area by these two methods is needed to determine the population structure of DBM within that area. Additionally, the source locations of DBM from transplants or migrations should be determined so that information on the susceptibility of incoming DBM can be examined.

In areas where there is an endemic population which is already resistant to one or more classes of insecticides, the outlook for insecticide resistance management is disquieting. Sun et al. (1986) indicate that pyrethroid resistance does not decline rapidly after the application of this class of insecticides is terminated. With organophosphorus, resistance may be more unstable (Sun et al. 1986), but exploiting this lack of stability requires other options which can be used to economically and effectively manage DBM in the field. In the US, *B. thuringiensis* has been a viable option, but our data, along with data from the Philippines (Kirsch and Schmutterer 1988) and Hawaii (Tabashnik et al. 1990) indicate DBM resistance to *B. thuringiensis*. With this information, the success of continued spraying or incorporating the *B. thuringiensis* gene into plants is questionable. Other compounds with unique modes of action are being developed, but their long-term usefulness depends on careful management, and that feasibility is also questionable. One example is the development of benzoylphenylureas (BPUs) which interfere with chitin synthesis. Unofficial reports from Thailand indicate that DBM has already developed significant resistance to several BPUs only 2-3 years after their introduction (Perng

et al. 1988). Development of noninsecticidal options such as cultural controls, biological control, pheromone disruption, sterile insect techniques and host plant resistance are urgently needed to allow insecticide resistance management strategies to be developed.

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Insecticide Resistance Characteristics of Diamondback Moth

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Abstract

Resistance to organophosphorus, carbamate and pyrethroid insecticides in the diamondback moth, *Plutella xylostella* (L.) is widespread in Japan. Consequently, tertiary amines such as cartap and thiocyclam, microbial insecticides (formulations of *Bacillus thuringiensis* Berliner) and chitin synthesis inhibitors have been widely used for control. A high level of resistance to these alternative insecticides has been observed. Organophosphorus insecticide resistance gradually develops by insecticidal selection. The resistance is rather unstable, but its stability tends to increase with resistance levels. Cross-resistance to organophosphorus insecticides is related to chemical structure of insecticides. Development of pyrethroid resistance is extremely rapid in the field and is more stable than organophosphorus insecticide resistance. Pyrethroid-resistant DBM exhibits high levels of resistance to various kinds of pyrethroids. A high level of resistance to tertiary amines and *B. thuringiensis* was induced in the field with successive applications, although development of the resistance is not fast. Tertiary amine resistance is more stable than *B. thuringiensis* resistance. *Bacillus thuringiensis* resistance decreased from a high to moderate level in a few generations in the absence of insecticide selection pressure during rearing.

Introduction

The damage to crucifers by the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), has gradually increased in Japan since 1960. Initially, it was easily controlled by organophosphorus insecticides such as dichlorvos. In the mid 1970s the effectiveness of several of these chemicals started declining at various locations. In the 1980s high resistance to various kinds of organophosphorus and carbamate insecticides had developed in southwestern Japan (Yamada 1979; Hama 1983; Sakai 1986).

Pyrethroids introduced to Japan in 1983 against this resistant moth were highly effective. However, high resistance to these pyrethroids was observed within only a few years (Makino and Horikiri 1985; Hama 1986a, 1987).

Tertiary amines such as cartap and thiocyclam, microbial insecticides (formulations of *Bacillus thuringiensis* Berliner) and chitin synthesis inhibitors have subsequently been widely and frequently used for control. Signs of resistance to these alternatives are already being observed. This ability to develop resistance to insecticides should be considered in planning applications of current and future alternative controls.

In this paper, resistance characteristics and application methods of the various insecticides that can delay this development are discussed.

Organophosphorus Resistance

Development and stability of resistance

As shown in Fig. 1, resistance to organophosphorus insecticides could gradually develop even in susceptible strains by selecting with organophosphorus insecticides for several generations (Sasaki 1982; Cheng et al. 1985; Noppun et al. 1986; Hama 1989b), whereas resistance has decreased through generations not subjected to insecticidal pressure (Chen and Sun 1986; Miyata et al. 1986; see M strain shown in Fig. 2). These characteristics differ among populations tested (Hama 1989b). When formerly organophosphorus-resistant strains, which have decreased resistance levels to organophosphorus insecticides from rearing without insecticidal pressure, were reselected with organophosphorus insecticides, they developed resistance faster than susceptible strains (Fig. 1).

In an earlier study (Hama 1988a), I observed that stability of organophosphorus resistance tends to increase with resistance levels. Moderate resistance to organophosphorus insecticides decreased drastically within only a few generations after collection, whereas decreases in high levels of resistance needed 10-20 generations or more.

Cross-resistance

According to results of selection experiments (Sasaki 1982), cross-resistance to organophosphorus insecticides is related to chemical structure of insecticides. When thiono-type

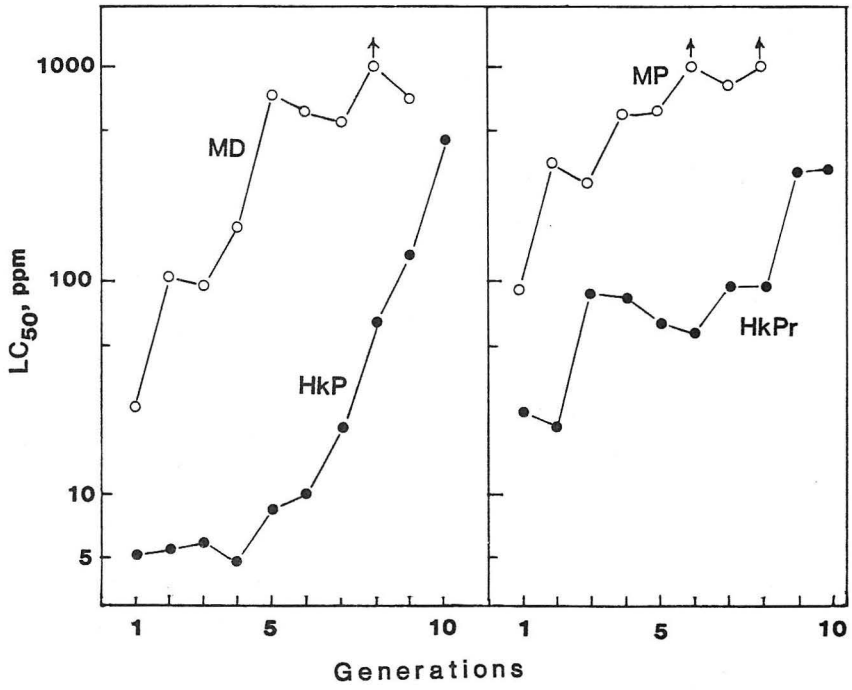

Fig. 1. Changes in LC_{50} values of organophosphorus insecticides used as selecting agents for four strains of DBM. MD, MP = strains derived from organophosphorus-resistant M strain by selecting with dimethylvinphos and phenthoate, respectively. HkP, HkPr = strains derived from a susceptible strain (Hk) by selecting with phenthoate and prothiophos, respectively. Arrows indicate values over 1000.

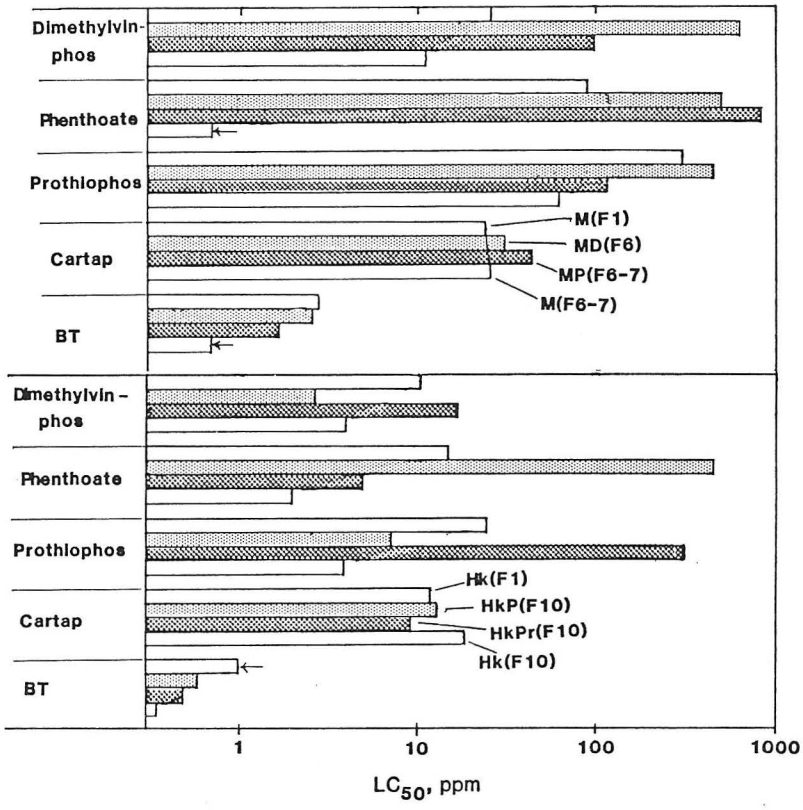

Fig. 2. LC_{50} values of five insecticides for six strains of DBM. For each strain, see footnotes in Fig. 1. Arrows indicate values under 1. BT = *Bacillus thuringiensis*.

insecticides were used as selecting agents, resistance level was higher than that to phosphate or dithio-types, although susceptibility to phosphate or dithio-types decreased. In a survey of organophosphorus insecticide resistance in field populations collected throughout Japan, the resistance level to thiono-types such as cyanofenphos, prothiophos, cyanophos and isoxathion tended to be higher than that to phosphate-type dimethylvinphos or dithio-type methidathion and phenthoate (Hama 1986b).

Cross-resistance to organophosphorus insecticides differs among populations. Two selected strains (MD and MP) derived from a formerly organophosphorus-resistant strain (M) exhibit a high level of resistance not only to selection agents, but to other organophosphorus insecticides. Resistance to organophosphorus insecticides in susceptible strains (HkP and HkPr) was limited to selecting agents (Hama 1989a; Fig. 2). All four selected strains did not show changes in their susceptibility to cartap and *B. thuringiensis*. This tendency of cross-resistance in DBM has also been demonstrated by Noppun et al. (1987) using phenthoate as a selecting agent.

Pyrethroid Resistance

Development and stability of resistance

Pyrethroid insecticide fenvalerate was introduced to Japan in 1983, and was highly effective in controlling DBM. It has therefore been in widespread use as an alternative to organophosphorus

insecticides in many areas. Soon thereafter, high resistance to this insecticide began to occur in many locations in the southeastern part of Japan (Hama 1987, 1989a; Fig. 3). In these locations, pyrethroids were used more than 10 times before resistance developed. Compared with organophosphorus insecticide resistance, pyrethroid resistance development was extremely rapid in the field, although its genetic behavior was incompletely recessive (Liu et al. 1981; Hama 1989a). It has been suggested that insecticide resistance can be suppressed by immigration of susceptible individuals (Georghiou and Taylor 1977). Therefore, probable causes of fast development of resistance might be: (1) a pyrethroid resistance gene(s) might have already been present frequently in the population when the pyrethroid was introduced; (2) migration of susceptible individuals was very limited in the areas where DBM is present throughout most

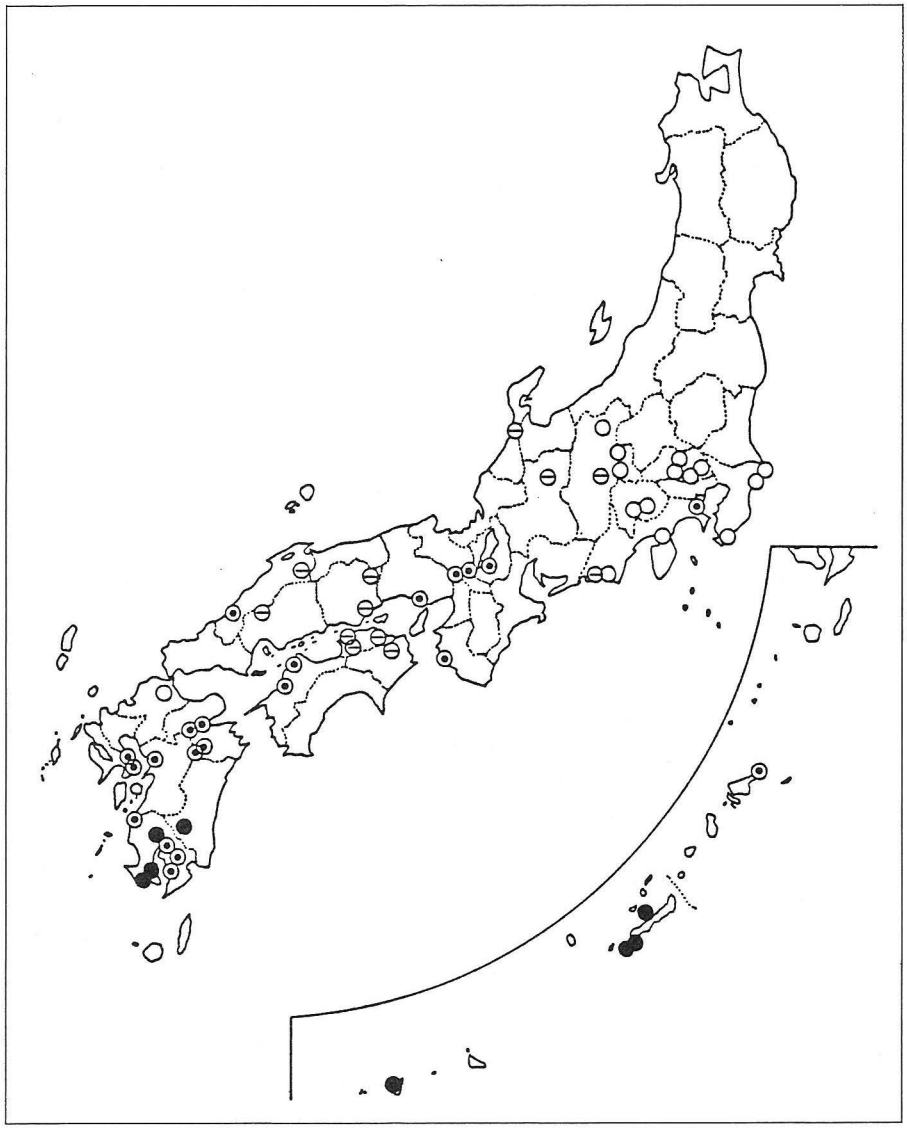

Fig. 3. Distribution of high levels of pyrethroid resistance in Japan. The pyrethroid-resistant moths were detected in 1984 (●), 1985 (⊙), 1986 (⊖), and 1987 (○).

of the year; and (3) such rather closed populations had been subjected to heavy applications of pyrethroids (Hama 1989a).

Pyrethroid resistance in DBM is more stable than organophosphorus resistance (Chen and Sun 1986). I have observed that although a high resistance level in a few populations decreases within 10 generations after collection, in most cases it remains for more than 15 generations (Hama 1988b).

Cross-resistance

Pyrethroid-resistant populations have exhibited high resistance to all pyrethroids when tested (Liu et al. 1981, 1982; Chou and Cheng 1983; Chen and Sun 1986; Hama 1987). This is in contrast to organophosphorus insecticide resistance, in which cross-resistance depends on chemical structures of insecticides, as mentioned above.

Alternative Insecticide Resistance

Tertiary amine resistance

Cartap has been widely used for the past 20 years in Japan, however, high levels of resistance to this tertiary amine has rarely occurred (Sakai 1986). Although LC_{50} values of cartap for field populations in Japan in 1980-82 were from 22 to 313 ppm (Sakai 1986), LC_{50} values of cartap for field populations recently collected in the southwestern part of Japan were 500-1000 ppm (Morishita and Azuma 1987; Horikiri and Makino 1987; Horikiri 1989; Ozawa et al. 1989). Such high values of cartap are considered to be the result of frequent applications of cartap in the field.

In Kohno (Osaka Prefecture) populations that exhibited a high level of resistance to *B. thuringiensis* (see below), LC_{50} value of cartap was 1100 ppm (Hama et al. 1990; see Fig. 4). This population also showed the same high level of resistance to thiocyclam, which had not been used. High levels of resistance to cartap and thiocyclam in the Kohno population remained for more than 10 generations in the absence of insecticidal pressure (Hama et al. unpublished data) as shown in Fig. 5.

B. thuringiensis resistance

Although *B. thuringiensis* had been introduced to Japan only in 1981 and used as alternative insecticides, high levels of resistance to this insecticide have not been found in fields. No records for *B. thuringiensis* resistance in insects have been reported in the 30 years since its introduction for the control of insect pests in the world, except in a lepidopteran pest of stored grain products *Plodia interpunctella* (Hübner) (McGaughey 1985).

Bacillus thuringiensis resistance in DBM was recently noted in Japan. LC_{50} values of *B. thuringiensis* for some field populations in the southwestern part of Japan, determined by leaf-dip method using 3rd instar larvae had increased from approximately 1ppm or below to 20-30 ppm with resistance ratio of 10-50-fold since the fall of 1986 due to selection (Morishita and Azuma 1987; Adachi et al. 1990). Besides, extremely high levels of resistance were found in 1988 in watercress (*Nasturtium officinale* R. Br.) populations. Watercress is cultured throughout the year in hydroponics in greenhouses at Kohno in Kishiwada City, Osaka Prefecture (Tanaka and Kimura 1990; Hama et al. 1990; see Fig. 4). *Bacillus thuringiensis* has been applied here 30 or 40 times in the past 3-4 years.

Bacillus thuringiensis Toarrow CT concentration-mortality lines of offspring derived from crossing of *B. thuringiensis* resistant (Kohno) and susceptible strains indicate that resistance in the moth is controlled mainly by an incompletely recessive, autosomal single allele (Hama et al. unpublished data).

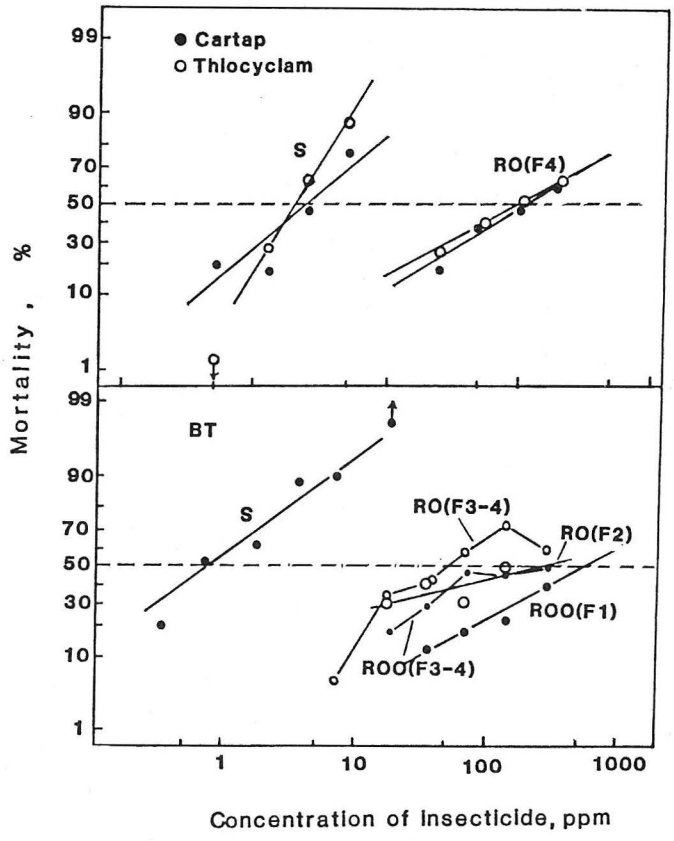

Fig. 4.
Concentration-mortality lines of tertiary amine (cartap and thiocyclam) and *B. thuringiensis* (Toarow CT) for Kohno populations and susceptible S strain of DBM. R0 and R00 populations were collected in watercress at Kohno of Kishiwada City in June and August of 1989, respectively. Arrows indicate 0% or 100%.

These extremely high levels of resistance to *B. thuringiensis* have decreased significantly (Fig. 5) within generations, LC_{50} value changing from more than 100 to 10 ppm reared in the absence of insecticidal pressure (Hama et al. unpublished data). Moderate levels of *B. thuringiensis* resistance in populations are apt to recover in the fields where this insecticide has not been used (Morishita 1990; Adachi 1990) and therefore seems to be unstable.

Bacillus thuringiensis concentration-mortality lines for moderately resistant field populations and highly resistant Kohno populations, appear successive. There appears to be no difference in quality of *B. thuringiensis* resistance among then.

The Kohno population exhibited high levels of resistance to two other *B. thuringiensis* subsp. *kurstaki* (Hama et al. 1990).

Chitin synthesis inhibitor resistance

Chitin synthesis inhibitor was more recently introduced into Japan (autumn 1988). Only slight resistance has been observed. Mortalities of some field populations in Okinawa Island, which were treated by leaf-dip method with the usual concentration of chlorfluazuron (25 ppm), were under 20-30%. The mortality of the majority of populations tested was over 90% (Tamaki 1990).

Conclusions

DBM has developed high levels of resistance to a wide range of insecticides, leaving few available insecticides for effective control. However, in many areas some organophosphorus

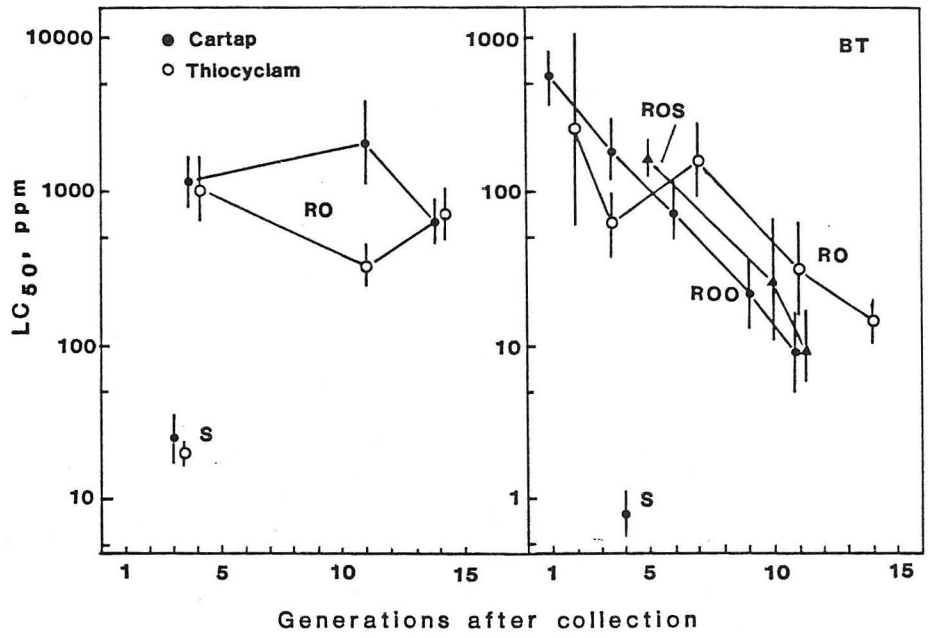

Fig. 5. Changes in LC_{50} values of tertiary amines (cartap and thiocyclam) and BT (*B. thuringiensis*) (Toarow CT) for Kohno populations that were reared in the absence of insecticidal pressure. For populations except for ROS which is R0 subjected to selection with *B. thuringiensis* in fourth generation, see footnotes in Fig. 4. Vertical bars indicate 95% fiducial limits.

insecticides such as dimethylvinphos and phenthoate still remain as effective as other groups of insecticides: tertiary amines, *B. thuringiensis* and chitin synthesis inhibitors. Mixtures of organophosphorus insecticides such as salithion or phenthoate and carbamate carbaryl are also effective in many areas (Kitamura and Nakazawa 1988; Kinumaki et al. 1989; Ozawa et al. 1989).

Even in areas where resistance to organophosphorus insecticides has spread, systemic chemicals such as acephate, carbosulfan or benfuracarb exhibit high effectiveness when their granules were applied with a hole-pricking or plant foot treatment at the transplanting stage (Horikiri and Makino 1987; Horikiri 1989).

In order to delay the rapid development of insecticide resistance, a rotational application of various groups of insecticides is recommended (Georghiou 1980). They should differ in function and resistance mechanism. In the context of characteristics of insecticide resistance as mentioned above, organophosphorus insecticides, tertiary amines and *B. thuringiensis* are recommended for rotational application, but pyrethroids are not. One study in the laboratory (Hama and Ando unpublished) showed that rotational application of *B. thuringiensis* or cartap with organophosphorus insecticides against moderately organophosphorus-resistant DBM did not seem to delay development of resistance to organophosphorus insecticides as compared with successive applications of organophosphorus insecticides.

From this result and characteristics of organophosphorus resistance, it is important to suppress the increase of resistance levels. For that we must change immediately an insecticide that has been used for another appropriate insecticide when any sign of resistance is detected.

In making a rotational system effective, the intervals between applications need to be long enough so that organophosphorus resistance fully disappears before that insecticide is reused. The intervals between insecticide applications in southwestern Japan are in fact usually very short. With such application regimes, it is very difficult to repress the rapid development of resistance even if a rotational application is employed.

We therefore suggest that a method of integrated pest management needs to be developed in order to reduce the number of insecticidal applications, incorporating rotational application with other techniques. These include avoidance of continuous growing of cruciferous plants, covering plants, introduction of cultivars resistant to the moth, and uses of sex pheromones, pathogens, parasites and predators.

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Resistance, Cross-resistance and Chemical Control of Diamondback Moth in Taiwan: Recent Developments

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Abstract

Except for the chlorinated insecticides, all traditional insecticides and newly introduced IGR-type compounds have been investigated for their modes of action in respect to the resistance in diamondback moth, *Plutella xylostella* (L.) in Taiwan. Diamondback moth has a very active, efficient, and inducible mixed function oxidases (MFO) system/ complex, which is responsible for the high level of resistance to carbamates, synthetic pyrethroids, and benzoylphenyl urea. Sublines of MFOs respond quickly to the selection pressure of a new type insecticide when its molecular structure fits the substrate for one of the many MFOs. So far, four sublines of MFOs have been found which are responsible for the resistance to common carbamates, carbofuran, all synthetic pyrethroids and benzoylphenyl ureas. The MFO-type resistance in DBM is the worst resistance for insecticides since it would respond indefinitely and render the insecticide useless at any dosage. By investigating the genetic background of different sub-lines of MFOs, a strategy of resistance management has been proposed for benzoylphenyl ureas due to the highly recessive nature of its genes. The same strategy cannot be applied to common carbamates and carbofuran since the responsible MFOs were not recessive. Tactics and strategies of DBM resistance management implementation are collectively discussed.

Introduction

Taiwan Agricultural Research Institute (TARI) initiated a research program on insecticide resistant diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera:Yponomeutidae), in 1980. The purpose was to rationalize the control strategy of this pest for implementation. Since 1985, two reviews on the resistance, cross-resistance and chemical control of DBM were prepared from the available literature. In the first review, sampling method, geographic distribution of resistance, resistance induction, and cross-resistance were carefully evaluated (Cheng 1986). The second review covered DBM resistance information from other Asia regions (Cheng 1988), which clarified the cross-resistance profile of different insecticide categories and suggested countermeasures. Up to 1988, most needed information concerning DBM resistance to traditional insecticides was available. The failure of insect growth regulators (IGRs) and the success of new *Bacillus thuringiensis* Berliner products represented recent developments in Taiwan. We are now assembling available DBM resistance management tactics for implementation.

IGR resistance

In 1987, the benzoylphenyl urea (BPU)-type IGRs were introduced into Taiwan for DBM control, and the possibility of IGR resistance has been of great concern because of sporadic reports on the IGR resistance in DBM in Thailand (Cheng 1988; Vattanatangum 1988). The DBM seems to possess a natural mixed function oxidase (MFO) with metabolic activity toward diflubenzuron and triflumuron (Cheng et al. 1988b), and a mild teflubenzuron resistance was induced in DBM by laboratory selection (Perng et al. 1988). The MFO degradation serves as the resistance mechanism for IGRs in houseflies and DBM (Pimprikar and Georghiou 1977; Lin et al. 1989). In Taiwan, sensitivity of field collected DBM to chlorfluazuron and teflubenzuron was mostly below 10 ppm (medium lethal concentration [LC_{50}]) before 1987 (Cheng et al. 1988). However, the susceptibility of Lu-chu DBM to chlorfluazuron had changed from 3.8 ppm in March to 924 ppm in December 1988, a 243-fold increase in resistance ratio (Cheng et al. 1990). By the end of 1989, DBM collected from five locations showed varied chlorfluazuron resistance as the LC_{50}s were 61.8-1685.7 ppm, while the sensitivities to teflubenzuron ranged from 100.5 to 72628.6 ppm (Cheng et al. 1990).

The IGR resistance observed in Taiwan DBM populations was significantly affected by the action of piperonyl butoxide. The synergistic ratios (S.R.) were 7.9-10.4 in three DBM populations for teflubenzuron (Cheng et al. 1990), while the S.R. in five IGR-resistant DBMs ranged from 2.6 to 16.3 for chlorfluazuron. The DBM collected from the Lu-chu area in December 1988 had a high IGR resistance, but the resistance declined from 7621- and 243-fold to only 6.5- and 3.1-fold for teflubenzuron and chlorfluazuron, respectively, after 17 selection-free generations (Cheng et al. 1990). The IGR resistance in DBM could be greatly reduced by crossing with the susceptible strain (Cheng et al. 1990). This genetic manipulation causes greater reduction in IGR resistance than incorporation of piperonyl butoxide in spray. Without further crossing for three more generations, the IGR resistance almost totally diminished in the offspring of RxS populations as the sensitivities to both teflubenzuron and chlorfluazuron were restored to that of the susceptible strain (Fig. 1), while the IGR selection-free F_1-F_7s of R-strain were still 78- and 20-fold more resistant to teflubenzuron and chlorfluazuron, respectively. The genetic expression of IGR resistance would be considered as highly recessive. By testing the backcross of F_1 to the susceptible and resistant strains as well as the F_2 offspring did not fit the suggested pattern of single gene control, which may be attributed to the heterozygosity of field-collected DBM used as the R-strain(Cheng et al. 1990). No evidence of sex-linked (Russell 1986) relationship in IGR resistance could be found in the reciprocal cross between susceptible and two IGR-resistant DBM strains (Cheng et al. 1990).

Evaluation of new *B. thuringiensis* products

In recent years, the improvement in *B. thuringiensis* efficacy has proceeded rapidly either by selecting mutants with higher potency or combining the *B. thuringiensis* toxins in a formulation (Moar et al. 1986, McGaughey and Johnson 1987; Padua et al. 1987; Gardner 1988; Morris 1988). For example, SAN415 was introduced in Taiwan in 1989 and its DBM control efficacy in the field was significantly better than other long-standing *B. thuringiensis* products (Chen 1990).

Some newly developed *B. thuringiensis* products were evaluated against three long-standing products, Thuricide, Bactospeine and Dipel in the field on their efficacies on DBM (Kao et al. 1990). Bactospeine gave the poorest results with 24.3-43.6% control, while Thuricide provided 43.8-49.5% control. On the contrary, new *B. thuringiensis* products are much more effective on DBM. For example, control efficacies of CGA237218 was 61.0% and Florbac FC and Florbac-XLV were 63.8 and 59.1-70.6%, respectively, while Bactospeine-XLV is an exception among new *B. thuringiensis* products with only minimum improvement in DBM control efficacy. The dosage used profoundly affected the control rate as the results of two CGA 237218 treatments indicated.

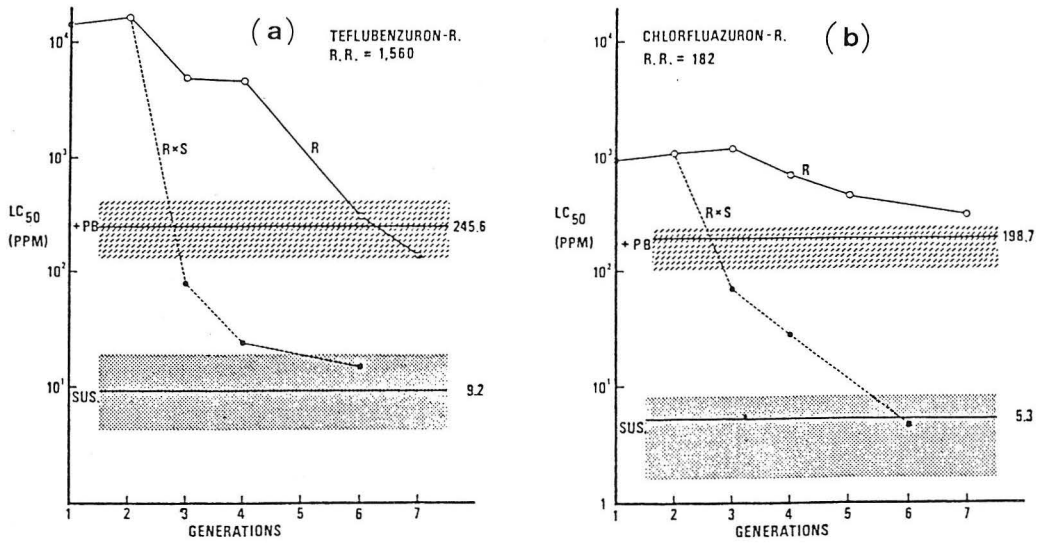

Fig. 1. The decline in resistance to teflubenzuron (a) and chlorfluazuron (b) (○) accerlerated by crossing with the susceptible-DBM (●).

▨ LC_{50}s for teflubenzuron and chlorfluazuron (averaged over three tests of F_1 to F_5 of SC-strain with addition of piperonyl butoxide) ranged from 444.8 to 128.4 ppm and 251.0 to 100.7 ppm, respectively.

▩ LC_{50}s for teflubenzuron and chlorfluazuron (averaged over 7 determinations of F_{59} to F_{77} of susceptible IL-strain) ranged from 19.55 to 4.47 ppm and 8.32 to 1.68 ppm, respectively.

Experimental chemicals

A. Diafenthiuron (Polo R)

A synthetic insecticide, diafenthiuron, gave extremely good control of both DBM and *Spodoptera litura*. The soluble concentrate (SC) formulation of diafenthiuron performed equally well for both pest species, while the wettable powder (WP) formulation was better on DBM than on *S. litura* (Kao et al. 1990). Diafenthiuron provided better and more stable control when compared to *B. thuringiensis* products. Since diafenthiuron was extremely effective against DBM in the field, its toxicity to both the susceptible and resistant DBM was further compared in the laboratory. Results showed that the multiple-resistant DBM had the same susceptibility (LC_{50}) to diafenthiuron as the susceptible strain, i.e. 245 ppm (Table 1), therefore, there is no cross-resistance from existing insecticides. The addition of piperonyl butoxide to diafenthiuron spray

Table 1. The susceptibility and synergistic tests of diafenthiuron on DBM for possible cross-resistance from other insecticides.

Insecticides or combinations	LC_{50} (ppm)		R.R. and S.R.[b]
	Suscep. strain	Resist. strain[a]	
Diafenthiuron	245.90	245.5	R.R. = 1
Diafenthiuron + piperonyl butoxide (1:5)	–	240.8	S.R. = 1

[a]Resistant SC-strain (Cheng et al. 1990). [b]Resistance ratio and synergistic ratio.

caused no difference, demonstrating that diafenthiuron is not affected by the inherited and induced oxidative metabolism in DBM.

Tridiphane (2-(3, 5-dichlorophenyl)-2-(2, 2, 2-trichloroethyl)oxirane, TDP)

It has been found that TDP can increase atrazine toxicity to weeds. Zorner and Olson (1981) showed that TDP inhibited atrazine metabolism in giant foxtail, and their preliminary data also indicated that TDP inhibited enzymatic conjugation of atrazine with GSTase. Similar synergistic effects of TDP on insecticides was observed in insects (Lamoureux and Rusness 1987). Diazinon metabolism in the housefly was inhibited by either or both TDP and S-(tridiphane) GSH and this was probably the cause of synergism of diazinon. By incorporating tridiphane as the synergist, approximately 2-fold increase in synergistic ratio for some OP insecticides was obtained in both the susceptible and resistant DBM strains (Tables 2 and 3). Since the action of TDP is so far not specific to R-strain, the role of GSTase in OP-resistance has not been confirmed, but the utilization of TDP in practical spray is worth investigating.

Table 2. The synergistic effect of TDP on two organophosphorus compounds in susceptible and resistant DBMs.

Insecticides	TDP synergistic ratio			
	Susc.	R1[a]	R2	R3
Mevinphos	1.9	1.7	2.1	1.7
Profenofos	1.3	1.5	2.6	1.8

[a]R_1, R_2, R_3 are three resistant strains.

Table 3. Synergistic ratios of TDP to several OPs in a resistant DBM strain.

Insecticides	TDP synergistic ratio
Mevinphos	1.7
Profenofos	1.8
Methidathion	1.6
Quinalphos	>2.0
Phenthoate	1.6
Parathion	1.8
Methamidophos	1.0
Diazinon	1.0
Mephosfolan	1.0

Update of DBM Resistance Mechanisms

1. MFO resistance

Resistance characteristics of DBM to different insecticides were extensively reviewed by Cheng (1988). With the additional information of IGR-resistance, oxidative metabolism obviously emerged as the most important detoxication mechanism in DBM. Chou and Cheng(1983) first noticed that DBM can develop carbofuran resistance, which acted independently from resistances of carbaryl, methomyl, synthetic pyrethroids and other insecticides. Hence, in the first International DBM Workshop, we objected to the term 'carbamate resistance' being used in connection with DBM. To settle the argument, Cheng et al. (1986) demonstrated the specificity of MFOs for different insecticides. New lines of MFO for metabolizing IGRs again confirmed the diversity of MFOs in DBM. We have now concluded that MFO is a single and

most important biochemical complex for the resistance problem in DBM. From the investigation of carbamates, synthetic pyrethroids, IGRs and other experimental insecticides, the characters of MFO resistance in DBM are outlined as follows:

(1) Naturally inherited MFO constituted the tolerance/resistance of DBM to certain insecticides such as carbaryl, methomyl and propoxur;
(2) MFO resistance to carbofuran, synthetic pyrethroids and IGRs are inducible;
(3) MFO possess a high diversity for different xenobiotics including experimental insecticides still being developed;
(4) MFOs are either specific for a single compound, for example, carbofuran, or a chemical group, i.e. synthetic pyrethroids; and
(5) MFOs are genetically diverse, i.e. highly recessive for IGRs and intermediate for synthetic pyrethroids and carbofuran, while inheritable for carbaryl, methomyl and propoxur.

All these facts indicate that DBM has a very efficient, active and either inherited or inducible MFO complex. Once a xenobiotic molecule fits the substrate for one of the many 'hidden' MFOs, DBM with this subline MFO would survive and propagate under the selection pressure. Hence a new type of MFO resistance appears. One general character of MFO-based resistance is that it would respond indefinitely in amplitude and eventually render the control chemical useless at any dosage. Carbaryl, methomyl, carbofuran, synthetic pyrethroids and IGRs have all met the same fate.

2. Glutathione-S-transferase involvement in organophosphorus resistance

Organophosphorus (OP) insecticide is one exception to the detoxication of MFO, and the OP resistance in DBM is multifactorial as described by Cheng (1988). Although the role of glutathione-S-transferase (GSTase) has been a controversial subject (Kao et al. 1989), the synergistic effect of tridiphane on mevinphos, profenofos, quinalphos, methidathion, phenthoate and parathion (Tables 2 and 3) provide positive evidence of GSTase involvement in the metabolism of OP insecticides; the OP-resistant DBM possessed higher GSTase activity (Cheng et al. 1983). Whether this detoxication is universal or specific is still under investigation.

3. A summation of important mechanisms in DBM insecticide resistance

The summation of important mechanisms are listed in Table 4.

Table 4. Important mechanisms in DBM insecticide resistance.

Resistance profile	OPs	Carbaryl, methomyl and propoxur	Carbofuran	Synthetic pyrethroids	IGRs	Cartap
Stability	stable to unstable	stable	unstable	unstable	very unstable	stable
Genetic expression	intermediate	dominate or inherited	intermediate	intermediate	recessive	additive (proposed)
Resistance mechanism involved	multifactorial	monofactorial	monofactorial	monofactorial	monofactorial	monofactorial (proposed)
Responsible biochemical entities	insensitivity AChE GSTase carboxylesterase esterase	MFOn[a]	MFOc	MFOs	MFOi	broad spectrum esterase (proposed)
Practical synergist	TDP	piperonyl butoxide	piperonyl butoxide	piperonyl butoxide	piperonyl butoxide	–

Table 4. Continued.

Resistance profile	OPs	Carbaryl, methomyl and propoxur	Carbofuran	Synthetic pyrethroids	IGRs	Cartap
Resistance amplitude	medium	high	high	high	high	mild
Comments for field usage	OPs with unstable resistance are useful in insecticide alternation program	Not recommended for use on crucifers;	Not recommended for used on fast-growing crucifers; occasionally used in early stage of crucifer to avoid residue problem	Strongly recommended for use in crucifers to control pests other than DBM	Can be used in a mosaic or alternation spraying program (still under development)	Recommended for use in an insecticide-alternation program except in seasons with high precipitation or dews due to its high water-solubility

[a]MFO_a, MFO_c, MFO_s and MFO_i are different sublines of MFO.

Proposed Tactics for DBM Resistance Management

1. Resistance monitoring

Since the use of insecticides for crucifers is unavoidable in Taiwan, the resistance monitoring becomes essential for constructing and implementing a rational DBM resistance management program. Currently, the DBM resistance to insecticides is monitored by the method developed by Cheng (1981), and the result is reliable and satisfactory although it is laborious and lengthy. A simpler leaf dipping method is under development for the large-scale resistance monitoring program. Insecticide solutions at 0.75-, 1- and 2-fold levels of the recommended dosages are used for dipping, and the mortality observations are set at 24, 48 or up to 120 hours for different insecticides. The result will either verify the efficacy of recommended dosage or indicate possible development of resistance to an insecticide.

2. Genetic dilution

The idea of using genetic dilution to combat resistance had been raised in a previous review (Cheng 1988). Resistance reduction by genetic dilution had been clearly demonstrated for carbofuran, fenvalerate and IGRs, and sometimes is more effective than the incorporation of synergist in spray. By utilizing the genetic dilution principle, two approaches, i.e., insecticide alternation and mosaic spray, are practical and possible.

A. Alternation of insecticides: Chemical insecticides should be alternated between groups with no or minimum cross-resistance as we recommended previously (Cheng 1988), and alternated with microbial insecticide such as *B. thuringiensis*. The reintroduction of any synthetic insecticide should be based on the results of resistance monitoring.

B. Mosaic spray management (proposed): The BPU-type IGR resistance in DBM is recessive and independent from other resistances. The IGR resistance of DBM in the field may be manageable if a sanctuary could be provided to harbor a portion of the DBM population free from the IGR selection. By mosaic spraying of IGR and *B. thuringiensis* in alternate rows, the dominant IGR-susceptible gene can be preserved in the field population. Genetic dilution would occur when the survivors of two different treatments mated, thus preventing or delaying IGR resistance. The proposed sanctuary in a mosaic spray should be designed in a way to provide maximum mixing for DBM adults, which in turn may depend on factors such as rate of dispersal.

The applicability of this strategy is currently being tested. Theoretically, mosaic sprays of IGRs and *B. thuringiensis* is desirable to minimize the possibility of producing cross-resistance, and both treatments also favor the survival of natural enemies. Preservation of natural enemies, sanctuaries for the dominant susceptible gene, and two insecticides free from cross-resistance should be considered as the most favorable combinations in an integrated pest management (IPM) scheme. If the IGR resistance continues to develop slowly in mosaic sprays of IGRs and *B. thuringiensis*, an insecticide such as mevinphos which causes only short-lived resistance (Cheng et al. 1985) can substitute as the control measure for a period of time to relieve the IGR selection. Two requirements are needed to conduct the mosaic spraying: (1) the farmers should be well educated and (2) the resistance should be closely monitored so that the needed adjustment can be made in time.

3. Synergists

Three major chemical groups of insecticides encountered oxidative metabolic resistance from at least four sublines of MFO. There are many synergists to counter the oxidative metabolism, and piperonyl butoxide is already in commercial use. The consequence of utilizing piperonyl butoxide to counter the resistance has not been fully evaluated in DBM, which can also become resistant to piperonyl butoxide (Chen and Sun 1986).

For some OP insecticides, the incorporation of tridiphane (TDP) resulted in a two-fold increase in control efficacy by inhibiting GSTase activity. It is still unclear how specific this synergist is to the OP resistance and physiological degradation. Some OP insecticides are still used in the alternation to manage the resistance, and the incorporation of this synergist in practice is worth consideration.

4. Natural enemies

Although the importance of parasitic wasps had been ignored before, their continual presence in cabbage fields still constitutes a natural mortality factor for DBM despite the heavy insecticide spray. Serious biological/ecological information gathering and the modelling between DBM and natural enemies are needed for an integrated resistance management program (IRMP). Besides, screening insecticides with minimum impact on natural enemies is also needed.

5. Farmers' education and participation

Farmers' education has nothing to do with the basic DBM resistance research, but is vital in implementing a DBM resistance management program. From the past experience, the traditional field demonstration tactic is a failure due to the free market practice of agrochemicals in Taiwan, which allowed farmers to receive both accurate and inaccurate information. The one pest, one chemical, one field and one morning demonstration session resulted in only minimal farmer involvement, and also was not realistic in dealing with the multiple pest situation of cruciferous fields.

A complete diagnosis of field pest problems, followed by proper recommendations to control DBM and other pests, are essential to gain the farmers' acceptance in future implementation programs. In other words, the extension agency cannot conduct a DBM resistance management program without incorporating the solutions for other important pests. Educational materials should also be aimed to protect the crucifers rather than to manage the DBM resistance only. It is also best to include the disease control information.

Farmer participation is another important element. Multiseasonal participation is needed, and the aim is to encourage the participating farmers to be future field instructors for others.

Constraints in DBM Resistance Management

The management of DBM resistance depends on rational and orderly use of insecticides, which usually is not possible since farmers use various insecticides to control other insect pests, thus interfering with DBM resistance management.

Geographical and seasonal differences in crop growth and pest abundance will affect the resistance management details.

Price fluctuations and the quality demands for vegetables seriously affect the farmer's commitment to IRMP.

The health hazard of pesticides to both farmers and consumers limits the insecticide selection and alternation.

Proposed DBM Resistance Management Strategy

Based on the above tactics and constraints, TARI has constructed a tentative strategy for the management of cruciferous pests with emphasis on DBM. This program which will last from July 1990 to June 1993, is carried out at two locations: Ten-chung and Pei-tou, with participation of six farms. It involves the following:

Monitoring the insecticide resistance in DBM. Insecticides screened were:

a. Permethrin (one synthetic pyrethroid is enough due to the cross resistance within the group).
b. Mevinphos, profenofos, methidathion, actellic (other OP compounds are not recommended due to their stable resistance).
c. Carbofuran (DBM has inherited tolerance to other carbamates).
d. Cartap.
e. Chlorfluazuron and teflubenzuron.

After analyzing the screening results, mevinphos (633 ppm) and cartap (833 ppm) were chosen in the insecticide alternation program.

Incorporating the newly developed *B. thuringiensis*: SAN 415 which is more effective than older products and acceptable to farmers is used. Its use can reduce the selection pressure of chemical insecticides.

Selecting proper insecticides for controlling other important cruciferous pests: Bifenthrin (28/ppm) is chosen to control *Spodoptera litura*, *S. exigua*, *Trichoplusia ni* and *P. rapae*.

By combining the data from these three activities, an IRMP for cruciferous pests, with emphasis on DBM resistance, is constructed. Mevinphos, cartap, SAN 415 and bifenthrin were provided to six participating farmers for field comparison with their own control practices. Spray details were recorded for IRMP program analysis. No insect count is needed since the degree of satisfaction of farmers on their vegetable quality is of most concern. The discussion session is held once a month between farmers and researchers.

Establishing the residue monitoring system. Less-educated farmers may ignore the safety aspects and use pesticides shortly before harvesting, hence residue control is needed. In Taiwan, we have established pesticide residue rapid bioassay stations in more than 40 farmers' associations and wholesale markets to detect pesticide residues on vegetables (Chiu et al. 1991), and one station has participated in this program.

Field demonstration and implementation. The recommended IRMPs are conducted and constantly improved in experimental fields on a year-round basis for 3 years. Educational sessions for the participating farmers are held every 30 days to discuss not only the DBM but also the identification and control technologies of other pests as well. Currently, the four-insecticides package recommended in Ten-chung and Pei-tou experimental sites have received most favorable response, and the program will expand from 6 to 70 farmers in 1991.

One advantage of this practical training is to keep the farmers away from the influence of profit-oriented agrochemical retailers who have been playing a major role in incorrect pesticide usage in Taiwan.

Other farmer associations have easy access to inspect the IRMP experimental fields and can have discussions with the participating farmers at any time. Any interested farmer can start the IRMP practice with instruction from the extension service of a Farmers' Association or from existing IRMP participating farmers.

Recent R and D for DBM Resistance Management

Two important scientific disciplines, genetics and biochemistry, have been brought together at TARI.

1. Selecting homozygous resistant strains for various insecticides: The OP insecticides are important in DBM resistance management programs since some of them are recommended in the alternation list. Since OP resistance is multifactorial, selection of genetically pure lines (monofactorial in biochemistry) with only one resistance mechanism hopefully will help to clarify the importance of individual mechanisms. The selection has been underway for more than a year now at TARI and the selection is quite successful. Similar progress has been obtained for carbofuran-resistant strain selection. However, the selection for synthetic pyrethroids and IGR-resistant strains was extremely difficult due to their recessive genetic nature. The overall results of various selections are similar to previous laboratory selections from the susceptible strain (Cheng et al. 1985 and 1988b; Chou and Cheng 1983).

2. Clarifying responsible biochemical entities, and developing biochemical detection kits for resistance: Biochemistry is fundamental to the study of toxicology, resistance mechanisms and the action of synergists, etc. A better understanding of the biochemical reaction will not only help to identify the resistance mechanism and sort out relationships of cross-resistance, but also facilitate the development of a rapid detection kit for resistance in the field. So far, the possible entities involved in DBM resistance are acetylcholinesterase, carboxylesterase, GSTase, MFOs, etc. Among them, GSTase has been a research subject in TARI for the past few years, and the effort has been concentrated on the purification and identification of various GSTase isozymes. Affinity chromatography has been used successfully to purify a major GSTase (Cheng et al. 1988a), two to three minor GSTases have been subsequently isolated by electrophoresis, and immunological investigation of GSTases has been initiated as well. AChE insensitivity test for different OP insecticides has also been initiated according to the method of Moores et al. (1988).

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Inheritance of Resistance to Phenthoate and Fenvalerate in Diamondback Moth and Management of Insecticide Resistance

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Abstract

The mode of inheritance of phenthoate and fenvalerate resistance in diamondback moth (*Plutella xylostella* (L.)) was incomplete dominant and incomplete recessive, respectively. Neither phenthoate resistance nor fenvalerate resistance was sex linked. Phenthoate and fenvalerate resistance was influenced by polygenic backgrounds. From results of mechanisms of phenthoate and fenvalerate resistance in diamondback moth, the management of resistance to insecticides is discussed. To retard or avoid the development of insecticide resistance, reduction of the selection pressure by the same insecticide is important. The rotational use of insecticides which do not show cross-resistance will be effective. The introduction of a combination of insecticides with negatively correlated cross-resistance will also be effective.

Introduction

Although resistance to various organic insecticides in the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) has been extensively documented (Sudderuddin and Kok 1978; Miyata et al. 1986). Little is known about the resistance mechanism and genetic basis for resistance (Liu et al. 1981; Hama 1989; Tanaka and Noppun 1989). Liu et al. (1981) and Hama (1989) found that the inheritance of fenvalerate resistance was controlled by autosomal incomplete recessive gene(s), and it was influenced by some polygenic backgrounds.

Noppun et al. (1986a, 1987b) obtained high levels of resistance to phenthoate and fenvalerate in DBM by successive selections in the laboratory. They found that phenthoate resistance in DBM is based on at least two mechanisms: (1) an efficient system of reduced cuticular permeability (Noppun et al. 1987e), and (2) an increased insensitivity of acetylcholinesterase (Noppun et al. 1987b). They found also that fenvalerate resistance is based on at least three mechanisms: (1) an efficient system of reduced cuticular permeability (Noppun et al. 1989b), (2) increased degradation of fenvalerate (Noppun et al. 1986b), and (3) an increased insensitivity of the nervous system (Noppun et al. 1986b). Therefore genetic studies were conducted to determine the mode of inheritance of phenthoate and fenvalerate resistance in DBM. From data obtained in this study and data obtained previously by us, we will discuss ways to manage the development of insecticide resistance in DBM.

Materials and Methods

Insects strains and maintenance

An experiment was conducted with two phenthoate-resistant (OKR-R and OSS-R), two phenthoate-susceptible (OKR-S and OSS-S), two fenvalerate-resistant (OKR-FR and KAR-FR) and two fenvalerate-susceptible (OKR and KAR) strains.

Phenthoate-resistant strains were those previously selected for resistance with phenthoate from the susceptible OKR and OSS strains, respectively (Noppun et al. 1986a). Two phenthoate-susceptible strains were selected for susceptibility with phenthoate from the susceptible OKR and OSS strains, respectively (Noppun et al. 1987d). Two fenvalerate-susceptible (OKR and KAR) strains which have been maintained without exposure to insecticides after collection were used for selection in the laboratory (Noppun et al. 1987c). Fenvalerate-resistant strains were those previously selected for resistance with fenvalerate from OKR and KAR strains (Noppun et al. 1987c)

The insects were mass-reared and maintained in a controlled room (25% RH, 16L:8D) as previously described (Noppun et al. 1983). The third instar larvae weighing an average 2.55 mg from the population were used for toxicity experiments.

Continued selection

The phenthoate-resistant (OKR-R and OSS-R) strains were further selected for homogeneous resistance to phenthoate by the method reported by Noppun et al. (1986a) for the successive 12 generations. In this selection 6 ml of phenthoate solution was used instead of 3 ml in the previous selection (Noppun et al. 1986a) because spraying with 3 ml of phenthoate solution did not result in more than 50% mortality at 24 hours. The phenthoate-susceptible (OKR-S and OSS-S) strains were also selected to obtain more homogeneous susceptibility to phenthoate by the method reported by Noppun et al. (1987d) for the successive three generations.

Genetic study

F_1 crosses were made in each strain by mass-mating of more than 100 adults of each sex according to the standard method reported by Georghiou (1969). To ensure the use of virgin insects, each pupa was isolated for eclosion individually in a test tube.

The degree of dominance (D) of resistance in F_1 offspring was calculated by Falconer's formula (1964) according to Stone (1968):

$$D = \frac{2LD_{50}(RS) - LD_{50}(RR) - LD50(SS)}{LD_{50}(RR) - LD_{50}(SS)}$$

where RR, RS and SS represent the resistant, heterozygote and susceptible populations, respectively.

Males and females of F_1 progeny were backcrossed to resistant (RR) females and to susceptible (SS) males, respectively. F_1 progeny were also allowed to intercross with F_2 progeny. The expected dose-response curve of the backcross progeny, assuming monofactorial inheritance, was calculated as followed (Georghiou 1969):

(1) for backcross progeny to SS or RR parents:

$$Xy = W_{(SR)}0.50\ W_{(SS\ or\ RR)}0.50$$

(2) for F_2 progeny:

$$Xy = W_{(SS)}0.25\ W_{(SR)}\ 0.50\ W_{(RR)}0.25$$

where X = the expected response at a given concentration y; and W = the observed response of SS, SR and RR genotypes at concentration y, obtained directly from the respective regression lines.

The single-gene hypothesis was tested by exposing the F_2 and the backcross progeny to a full range of concentrations of phenthoate and fenvalerate, respectively. If a single gene is responsible for insecticide resistance then plateaus will occur in the F_2 regression line at about 25 or 75% mortality. Goodness of fit of observed to expected mortalities was tested by Chi-square analysis and the t distribution at 95% confidence limits.

Toxicity tests

The third instar larvae were topically applied with 0.52 μl of acetone solution of insecticide. The details of a standard method are given in Noppun et al. (1983). However, for the genetic study 20 larvae were used at one dose level with three replications, instead of 10 larvae in the standard method. At least nine dose levels of insecticide were employed and mortalities at 24 hours after topical application were taken and subjected to probit analysis (Finney 1971) using a microcomputer (NEC PC-9801VX, NEC Co. Ltd., Tokyo).

Results and Discussion

Continued selection

The continued selections of the phenthoate-resistant (OKR-R and OSS-R) strains for 12 generations resulted in a further increase in resistance levels. After the last selection, the OKR-R and the OSS-R strains showed higher LD_{50} values to phenthoate (48,400 and 44,800 μg/g) than those reported previously (16,200 and 13,600 μg/g) (Noppun et al. 1986a), respectively. On the other hand, continued selection of the phenthoate-susceptible (OKR-S and OSS-S) strains (Noppun et al. 1987d) for more susceptibility with phenthoate for three generations did not give a further increase in phenthoate susceptibility.

Genetic study

Results of toxicity tests with phenthoate against the parental and hybrid populations are given in Tables 1 and 2. Resistance to phenthoate of the F_1 progenies of OKR-R × OKR-S and OKR-R × OKR-S crosses had fallen in the mid-parent range (D of LD_{50} and LD_{95} values = 0.15 and 0.15, and 0.47 and 0.55, respectively). Similarly, resistance of the F_1 progenies of OSS-R × OSS-S and OSS-R × OSS-S crosses to phenthoate was also in the mid-parent range (D of LD_{50} and LD_{95} values = 0.15 and 0.11, and 0.50 and 0.32, respectively). When

Table 1. Susceptibility to phenthoate in the phenthoate-resistant (OKR-R) strain, the phenthoate-susceptible (OKR-S) strain and various interstrain crosses.

Strains	SRE[a]	LD_{50} (μg/g)	RF of[b] LD_{50}	D of[c] LD_{50}	LD_{95} (μg/g)	RF of[d] LD_{95}	D of[e] LD_{95}
OKR-R (R)	1.95	48400	1870	–	338000	2000	–
F11 (R × S)	1.21	1950	75.3	0.15	44500	265	0.47
F12 (R × S)	1.10	1980	76.5	0.15	61100	364	0.55
F12 × R	1.11	14800	571	–	448000	2660	–
F12 × S	0.87	490	18.9	–	38200	227	–
Intercross F11	0.84	1290	49.8	–	116000	690	–
Intercross F12	1.20	1070	41.3	–	25300	151	–
OKR-S (S)	2.03	25.9	1.0	–	163	1.0	–

[a]Slope of regression equation. [b]Resistance factor of LD_{50} = LD_{50} of RR or RS population/LD_{50} of SS population. [c]Degree of dominance of LD_{50}. [d]Resistance factor of LD_{95} = LD_{95} of RR or RS population/LD_{95} of SS population. [e]Degree of dominance of LD_{95}.

Table 2. Susceptibility to phenthoate of the phenthoate-resistant (OSS-R) strain, the phenthoate-susceptible (OSS-S) strain and various interstrain crosses.

Strains	SRE[a]	LD_{50} ($\mu g/g$)	RF of[b] LD_{50}	D of[c] LD_{50}	LD_{95} ($\mu g/g$)	RF of[d] LD_{95}	D of[e] LD_{95}
OSS-R(R)	1.96	44800	1200	–	309000	1410	–
F_{11} (R♂ × S♀)	1.21	2180	58.3	0.15	49400	226	0.50
F_{12} (R♀ × S♂)	1.43	1920	51.3	0.11	26900	123	0.32
F_{12}♂ × R♀	1.35	9680	259	–	161000	735	–
F_{12}♀ × S♂	1.10	382	10.2	–	11800	53.9	–
IntercrossF_{11}	0.96	1670	44.7	–	87100	398	–
IntercrossF_{12}	0.98	1340	35.8	–	62900	287	–
OSS-S(S)	2.14	37.8	1.0	–	219	1.0	–

Footnotes as in Table 1.

D values are compared between two different crossings in each strain, the difference is small. Results from the present study revealed that inheritance of resistance to phenthoate in DBM was derived by more than one gene, incomplete dominant genes and no sex linkage. From studies on the mechanism of phenthoate resistance in DBM, it was also found that reduced cuticular penetration and reduced sensitivity of acetylcholinesterase are involved in the mechanism of phenthoate resistance (Noppun et al. 1987b, e).

The observed dose-response curves of the backcross progeny (F_{12}(R♀ × S♂))♂ × S♀), (F_{12} (R♀ × S♂)♀ × S♂) and intercrosses of F_{11} and F_{12} progenies differ significantly from the curves which are expected on the basis of monofactorial inheritance. Computation of the 95% confidence limits confirms the significant differences between the observed and expected curves except for a small region near LD_{50} (data are not shown). It is thus concluded that phenthoate resistance in DBM is influenced by polygenic backgrounds.

Results of toxicity tests with fenvalerate against the parental and hybrid populations are given in Tables 3 and 4. Resistance of the F_1 progenies of OKR-FR♂ × OKR♀ and OKR-FR♀ × OKR♂ crosses to fenvalerate had fallen in the mid-parent range (D of LD_{50} and LD_{95} values = −0.50 and −0.17, and −0.53 and −0.07, respectively). Similarly, resistance of the F_1 progenies of KAR-FR♂ × KAR♀ and KAR-FR♀ × KAR♂ crosses to fenvalerate was also in the mid-parent range (D or LD_{50} and LD_{95} values = -0.53 and -0.39, and -0.44 and -0.41, respectively). D values seemed to be different between two crossings in OKR-FR and OKR strains. Since the difference in the slope of regression equations is statistically nonsignificant, it is therefore concluded that there is no sex-linkage in the inheritance of fenvalerate resistance.

The observed dose-response curves of the progeny of the backcrosses, (F_{11}(R♂ × S♀)♂ × S♀), (F_{11}(R♂ × S♀)♀ × R♂), (F_{12}(R♀ × S♂)♂ × S♀) and (F_{12}(R♀ × S♂)♀ × R♂) and intercrosses of F_{11} and F_{12} progenies differ significantly from the curve which might be expected on the basis of monofactorial inheritance (data are not shown). Computation of the 95% confidence limits confirms the significance of differences between the observed and expected curves except for a small region near the LD_{50}.

It is thus concluded that fenvalerate resistance in DBM is due to more than one gene. Liu et al. (1981) and Hama (1989) also reported that fenvalerate resistance is inherited through partially recessive genes with no sex linkage. According to Noppun et al. (1986b, 1989a, b), fenvalerate resistance is controlled by at least three different mechanisms: (1) increased metabolism (esterase and mixed function oxidases), (2) reduced cuticular permeability, and (3) reduced sensitivity of the central nervous system.

Management of insecticide resistance

Yamada (1977) pointed to the following reasons for the increase in DBM damage: (1) year-round cultivation of crucifers, especially cabbage which is an excellent host plant; (2) increase

Table 3. Susceptibility to fenvalerate of the fenvalerate-resistant strain (OKR-FR), the fenvalerate-susceptible strain (OKR) and various interstrain crosses.

Strains	SRE[a]	LD_{50} ($\mu g/g$)	RF of[b] LD_{50}	D of[c] LD_{50}	LD_{95} ($\mu ug/g$)	RF of[d] LD_{95}	D of[e] LD_{95}
OKR-FR (R)	0.92	877	1620	–	53500	16200	–
F11 (R × S)	1.71	3.47	6.43	-0.50	31.9	9.67	-0.53
F12 (R × S)	1.16	11.3	21.7	-0.17	305	92.4	-0.7
F11 × R	0.64	9.18	17.0	–	3480	1050	–
F11 × S	2.38	0.787	1.46	–	3.86	1.17	–
F12 × R	0.57	15.1	28.0	–	12200	3700	–
F12 × S	2.45	0.966	1.79	–	4.54	1.38	–
Intercross F_{11}	0.87	0.013	0.02	–	0.774	0.23	–
Intercross F_{12}	1.01	0.014	0.08	–	1.73	0.52	–
OKR(S)	2.10	0.54	1.0	–	3.30	1.0	–

Footnotes as in Table 1.

Table 4. Susceptibility to fenvalerate of the fenvalerate-resistant strain (KAR-FR), the fenvalerate-susceptible strain (KAR) and various interstrain crosses.

Strains	SRE[a]	LD_{50} ($\mu g/g$)	RF of[b] LD_{50}	D of[c] LD_{50}	LD_{95} ($\mu g/g$)	RF of[d] LD_{95}	D of[e] LD_{95}
KAR-FR (R)	0.61	3330	5840	–	192000	53000	–
F_{11} (R × S)	1.33	4.36	7.65	-0.53	75.2	20.8	-0.44
F_{12} (R × S)	1.57	7.93	13.9	-0.39	88.1	24.3	-0.41
F_{11} × R	0.53	45.2	79.3	–	59600	16500	–
F_{11} × S	2.35	0.944	1.66	–	4.73	1.31	–
F_{12} × R	0.46	84.3	148	–	146000	40300	–
F_{12} × S	2.24	0.891	1.56	–	4.84	1.34	–
Intercross F_{11}	1.10	0.031	0.05	–	0.954	0.26	–
Intercross F_{12}	1.38	0.081	0.14	–	1.00	0.28	–
KAR (S)	2.04	0.57	1.0	–	3.62	1.0	–

Footnotes as in Table 1.

in the area of cabbage cultivation; and (3) insecticide resistance caused by frequent application of insecticides, especially in tropical countries where the number of DBM generations is more than 20 (Ho 1965; Sun et al. 1978).

To manage the insecticide resistance, one must (1) manage cultivation of crucifer crops; (2) introduce control methods that do not use insecticides to reduce the insecticide pressure; and (3) reevaluate conventional control methods that use insecticides. In this paper, only the third point will be discussed.

There are two ways to overcome the problem of development of insecticide resistance: (1) avoid or retard the development of insecticide resistance, and (2) control the insecticide-resistant DBM. The results of studies on the mechanisms of resistance to phenthoate and fenvalerate in DBM are given in Table 5. From these data, the rotational use of insecticides that have no cross-resistance will be important to reduce the selection pressure by the same insecticide. It would certainly be preferable to increase the time interval between applications of the same insecticide. The introduction of insecticide combinations that have negatively correlated cross-resistance will be effective, as reported in the rice-leaf and planthoppers by Miyata and Saito (1984). In DBM, chitin synthetase inhibitors seem to show negatively correlated cross-resistance with juvenile hormone analogs (Sinchaisri et al. 1990) as reported in *Spodoptera littoralis* (El-Guindy et al. 1983). Introduction of a new type of insecticide or the use of a synergist is effective to overcome insecticide resistance, however, more than two different resistance mechanisms are involved

in the resistance (Table 5), and it is not easy to increase the susceptiblity of the resistant strain to the level of the susceptible one with a synergist. If they are used extensively, DBM can develop resistance to them (Sun et al. 1986; Takeda et al. 1986).

According to the simulation study by Tabashnik (1986), DBM develops resistance to insecticides in a short period, so it is important to extend the application interval of the same insecticide as much as possible.

The insecticide resistance level is reported to decrease after relaxation of insecticidal pressure (Noppun et al. 1984; Sun et al. 1986; Hama 1989). Possible explanations for the phenomenon include: (1) some genetic variance in insecticide resistance among individuals, (2) some genetic variance of reproductive ability among individuals; and (3) a possible negative correlation between insecticide resistance and reproductive ability of an individual (Tsubaki et al. 1988). Development of simple monitoring methods for resistance is also necessary, especially methods to detect individual resistance levels (Miyata 1983; Miyata and Saito 1984; Miyata 1989).

Table 5. The mode of resistance to phenthoate and fenvalerate in DBM.

Phenthoate resistance	Fenvalerate resistance
Reduced cuticular penetration	Reduced cuticular penetration Increased metabolism
Increased sensitivity of AChE	Reduced sensitivity of CNS
High cross-resistance to: prothiophos, cyanophos and methomyl	High cross-resistance to: pyrethroids
	Cross-resistance to: phenthoate, prothiophos, cyanophos and methomyl
Low cross-resistance to: dichlorvos and cartap	
No cross-resistance to: acephate and fenvalerate	No cross-resistance to: cartap
Synergism with TPP	Synergism with TPP and PB
Unstable resistance	Unstable resistance

(Source: Noppun et al. 1984, 1986b, 1987a, 1987b, 1987e, 1989a, 1989b).

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INTEGRATED PEST MANAGEMENT

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Management of Diamondback Moth in Central America

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Abstract

In Central America, most information on diamondback moth, *Plutella xylostella* (L.), comes from CATIE in Costa Rica, the National Autonomous University of Nicaragua and the Pan American Agricultural School in Zamorano, Honduras. Assisted by international collaborators, the Zamorano Cabbage IPM Program has developed and implemented improved management practices for key pests and diseases including diamondback moth. Farmers motivated by repeated failures of costly chemical control participate in technology generation and transfer programs. Organophosphorus, carbamate and pyrethroid insecticide resistance levels in Honduras are variable but generally high. The Honduran IPM program makes use of cultural practices (especially irrigation) and microbial control using *Bacillus thuringiensis* Berliner. Farmers using the IPM program reduce applications of synthetic pesticides from nine to two per crop cycle. Pesticide residues in harvested cabbage are dramatically reduced. The program increases farmers' net income. Attempts to complement the action of the native parasitoid *Diadegma insulare* (Cresson) with imported exotics have not yet been successful. Neem provides effective larval control. *Autographa californica* (Speyer) NPV is promising. Glossy cabbage lines are tolerant to DBM.

Introduction

Diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), is a key pest of crucifers in Central American countries (King and Saunders 1984; Secaira and Andrews 1987); there is considerable concern among scientists, consumers and farmers regarding DBM management. Most published information comes from CATIE in Costa Rica, the National Autonomous University of Nicaragua, and the Pan American Agricultural School in Zamorano, Honduras. Very little literature is available from Guatemala despite the importance of crucifers in that country. The proceedings of three recent regional IPM conferences have yet to be published, but information from dozens of presentations on DBM management is included here. CATIE (1990) published a useful booklet on IPM in Central America on cabbage after this review was completed; readers should consult it for further information.

Resistance to pesticides has been documented in both Costa Rica and Honduras. Ovalle and Cave (1990) found that DBM larvae collected in Zamorano showed 45-, 224-, and 412-fold levels of resistance to methomyl, methamidophos and cypermethrin, respectively (Fig. 1). Resistance levels were somewhat lower in two other Honduran populations. Problems with residues in crucifers are discussed later.

In Honduras and the rest of Central America, cabbage is produced year-round. In some areas, it is produced only during the rainy season and fields are left fallow during the dry season. In other areas, cabbage is continuously cropped using irrigation during the dry season. It is produced primarily above 1000 m. In Honduras, plots range from small backyard gardens to

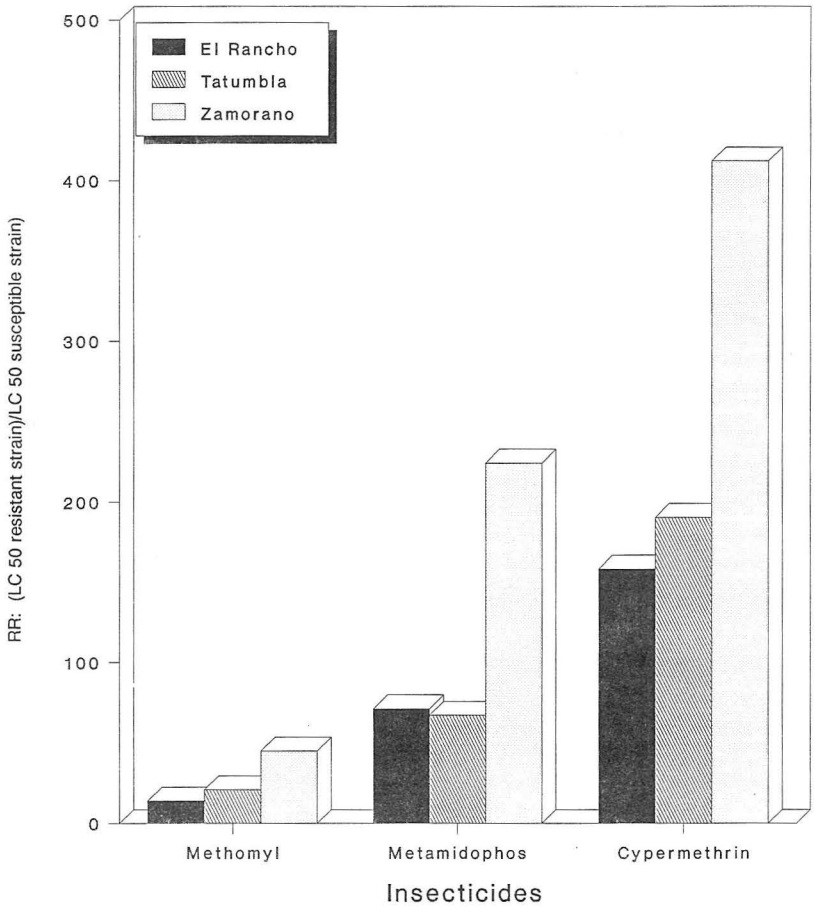

Fig. 1. Resistance levels of three Honduran populations of DBM larvae to commonly used insecticides as compared to a susceptible strain provided by NYSAES (after Ovalle and Cave in press).

about 2 ha. Average plot size is approximately 0.3 ha. Farmers who plant cabbage may be independent producers who finance themselves, or they may depend on credit. In most cases, a share-cropping arrangement (*mediania*) is used (Ardon and Sanchez 1990a). This special form of financing and growing cabbage has several important consequences for IPM technology generation and transfer which are discussed below.

Zamorano's 3-year-old crucifer IPM program focuses on simultaneous technology generation and implementation. Work on DBM is one of several lines of work; other organisms that receive emphasis include *Xanthomonas campestris*, limacid and veronicellid slugs, seedling diseases and pierid pests. The program makes extensive use of participative techniques for technology development and transfer. Ardon and Sanchez (1990b) showed that farmers make more than one-half of the intellectual contributions to studies of cultural control carried out by our research team. They make between one-third and one-half of the intellectual contributions to studies of supervised control, host plant resistance and chemical control. They make less than 15% of the total intellectual contribution to background studies of pest biology and ecology.

Natural Biological Control

Cordero and Cave (in press) reared three primary parasitoids from DBM larvae and pupae. Neither *Opius* sp. nor *Coccygomimus punicipes* (Cresson) appear to be important. *Diadegma insulare* (Cresson) accounted for 99% of the primary and facultative parasitoids reared. It was taken throughout the year and in all crucifer-producing areas of Honduras sampled. Cordero and Cave (1990) reported parasitization levels of 9-36% (mean = 23%) in insecticide-treated fields and 18-47% (mean = 29%) in untreated fields.

Two species of *Spilochaleis* are facultative hyperparasitoids, and 11 species of obligate hyperparasitoids attack *D. insulare* (Table 1). Three vespids, *Polybia diguetana* du Buysson, *Polybia occidentalis nigratella* du Buysson and *Brachygastra mellifica* (Say) are DBM predators (Cordero and Cave, in press). Natural enemies of eggs have not been studied. No naturally occurring epizootics affecting DBM have been observed in Honduras.

Table 1. Predators, parasitoids and hyperparasitoids associated with DBM in Honduras (Cordero and Cave 1990).

Primary Parasitoids	
1. *Diadegma insulare* (Cresson)	
2. *Opius* sp.	
3. *Coccygomimus punicipes* (Cresson)	
Facultative Hyperparasitoids	
1. *Spilochalcis pseudofulvovariegata* Becker	
2. *Spilochakis petioliventris* (Cameron)	
Obligate Hyperparasitoids	Predators
Isdromas lycaenae (Howard)	*Polybia diguetana* du Buysson
Mesochorus sp. 1	*Polybia occidentalis nigratella* du Buysson
Mesochorus sp. 2	*Brachygastra mellifica* (Say)
Trichomalus sp. 2	
Pteromalinae Genus species 1	
Pteromalinae Genus species 2	
Haltichella ornaticornis (Cameron)	
Spilochakis hirtifemora (Ashmead)	
Ceraphron sp.	
Epyris sp.	
Apenesia sp.	

Manipulation and Conservation of Natural Enemies

In addition to the use of *Bacillus thuringiensis* Berliner var. *kurstaki* and application of insecticides only as needed, we know of no work to preserve and enhance naturally occurring DBM parasitoids and predators. Farmers are encouraged not to destroy *Polybia* nests, and a few farmers even move *Polybia* nests to the edge of cabbage fields in an attempt to increase the effectiveness of these predators (Bentley 1990).

Classical Biological Control

Attempts to introduce the larval parasitoid *Cotesia plutellae* (Kurdjumov) into Honduras (see Waage and Cherry in this Volume), Belize and Costa Rica have not resulted in pest suppression. We have made occasional recoveries in the highlands surrounding Zamorano. We also released the pupal parasitoid *Diadromus collaris* (Gravenhorst) during 1990 in three sites; it was established at least temporarily in Zamorano and has been recovered in the highlands 20 km from a release site. Cordero and Cave (in press) expected that the hyperparasitoids recorded in their survey would not inhibit the establishment and potential effectiveness of the introduced species. Zamorano intends to continue its efforts in classical biological control in cooperation with the International Institute of Biological Control, the Caribbean Agricultural Research and Development Institute and other collaborators.

Microbial Control

Bacillus thuringiensis has rapidly become an essential component of programs to manage DBM in Honduras and other Central American countries (Fig. 2). In 1980, an unpublished government survey revealed that no Honduran farmers used it. In 1988, 12% relied exclusively on *B. thuringiensis* for DBM control and 34% either tank-mixed or rotated it with synthetic insecticides. By 1989, 24 and 28% relied exclusively or partially on *B. thuringiensis*. The efficacy and cost effectiveness of *B. thuringiensis* have been shown by Centeno (1990) in Nicaragua, Varela and Guharay (in press) in Nicaragua, Mora and Secaira (1989) in Honduras, Moncada and Sánchez (1990) in Honduras, and Cerna (1990) in Honduras. The Nicaraguan government is attempting to produce *B. thuringiensis* locally. A liquid formulation of *B. thuringiensis* is produced in Guatemala. In Honduras, Moncada and Sanchez (1990) showed that a Guatemalan commercial formulation of *Autographa californica* (Speyer) NPV controlled DBM larvae as well as Dipel and better than other synthetic insecticides (Fig. 3).

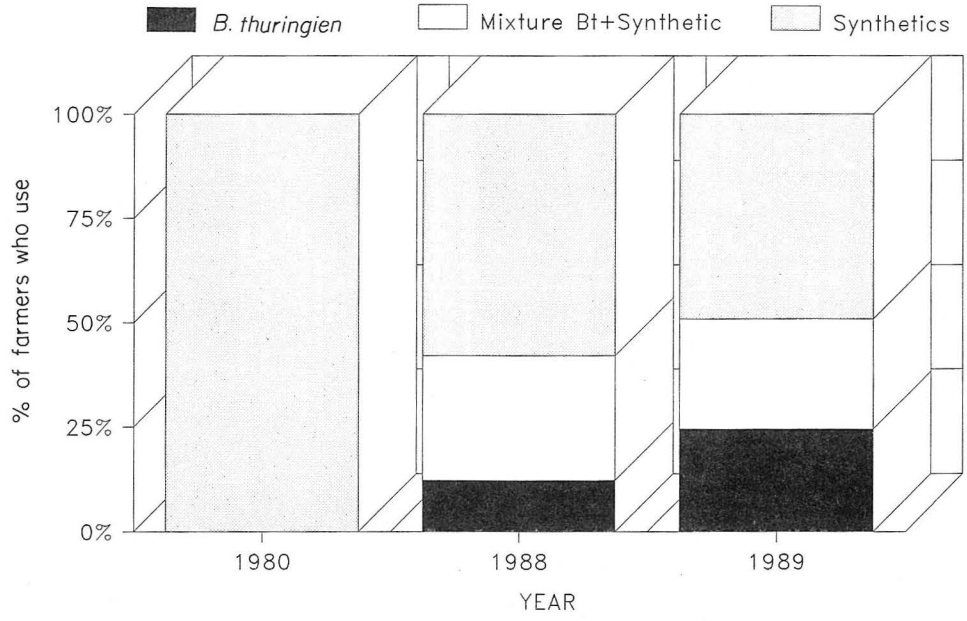

Fig. 2. *Bacillus thuringiensis*-based insecticides are replacing synthetic insecticides in Honduran cabbage. Unpublished data from interviews of 93, 57 and 60 producers in the Siguatepeque region.

Botanical Extracts

Extracts of plants with insecticidal properties are widely researched in Central America. In Nicaragua, 240,000 neem trees (*Azadirachta indica* A. Juss) are producing fruits. The previous Nicaraguan government sponsored a major research and development program to use aqueous extracts for control of key agricultural pests, including DBM (Barahona and Miranda 1990; Barahona 1990). In Honduras, Sánchez et al. (1990 a) and Moncada and Sánchez (1990) reported that aqueous extracts of neem provide effective control of DBM larvae (Fig. 3). These same researchers also found that the application of water extracts of onion, garlic and pepper, or old cabbage leaves reduced DBM larval populations somewhat compared to water only check.

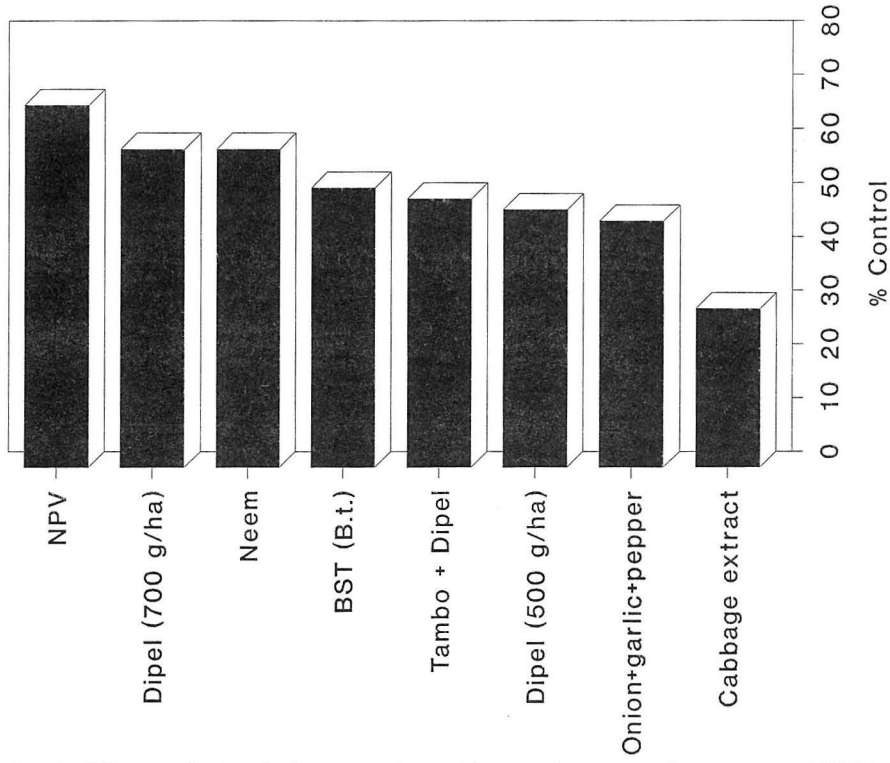

Fig. 3. Efficacy of microbial insecticides and botanical extracts for control of DBM in Honduran cabbage as compared to water only check (after Moncada and Sánchez 1990).

Chemical Control

Until recently, Honduran producers relied almost exclusively on synthetic insecticides for DBM control. Farmers applied on average six and ten times in the wet and dry seasons, respectively. Preferred products of the last 5 years such as fenvalerate, deltamethrin, cypermethrin, methomyl, carbaryl, methamidophos and mevinphos were shown by Portillo and Jimenez (1990), Navarro (1990), Mora and Secaira (1989), Moncada and Sánchez (1990), and Herrera and Secaira (1990) to be mostly ineffective in Honduras (Fig. 4). Tambo 440EC, a commercial mixture of cypermethrin (0.4 kg ai/ha) and profenofos (0.04 kg ai/ha), provided reasonable control and was sold until this year; it has now fallen out of favor with farmers. Costa Rican farmers make 16 calendarized applications (Carballo et al. 1989a). Most farmers

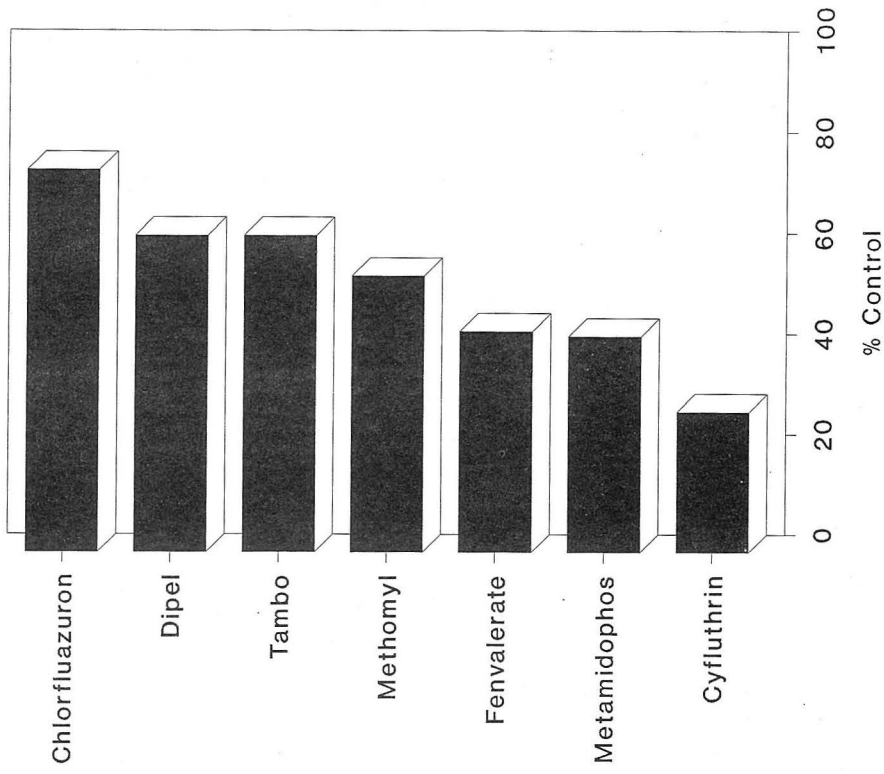

Fig. 4. Efficacy of synthetic insecticides for control of DBM in Honduran cabbage as compared to untreated check (after Moncada and Sanchez 1990).

apply using backpack sprayers and 300-400 l water/ha. Spraying during the afternoon gave the same control as at others times of the day (Chávez 1989). Mixtures of synthetic insecticides with microbials are increasingly common, as are rotations and tank mixes of *B. thuringiensis* and synthetic insecticides.

The current Zamorano extension program suggests that farmers should apply appropriate organophosphorus products until 20 days after transplant for DBM and cutworm control. Thereafter, we suggest preventive calendar applications of *B. thuringiensis* once weekly during the dry season and once every 2 weeks during the rainy season.

During the 1989 dry season and 1990 rainy season, Sánchez et al. (1990b) determined insecticide residues in 57 randomly selected lots of cabbage in Honduras. They detected 24 different insecticides mostly in trace quantities (Fig. 5); these residues appear to be low-level contaminants resulting from applications made in previous years. However, cypermethrin and chlordane residues averaged 5.5 and 4.3 ppm in the dry season, respectively. In the wet season these values dropped to 0.8 and 0.6 ppm, respectively. These unacceptably high residues are especially distressing considering that chlordane has not been registered for use on food crops for a decade. During the dry season 1990, residues of cypermethrin and chlordane on cabbage produced by farmers using the Zamorano IPM program were 0 and 0.3 ppm, respectively, while conventional farmers' cabbage contained 1.9 and 8.3 ppm. The high levels found cannot be explained as background contamination. Nor do the data support the idea that chlordane is being used consciously by a few misguided farmers since levels varied little from farmer to farmer. We are currently investigating the possiblity that a common commercial product has been adulterated during the formulation/distribution process. The Guatemalan broccoli export industry suffered a major setback in 1990 when the U.S. rejected large quantities of frozen broccoli found to be tainted by illegal residues of phenthoate.

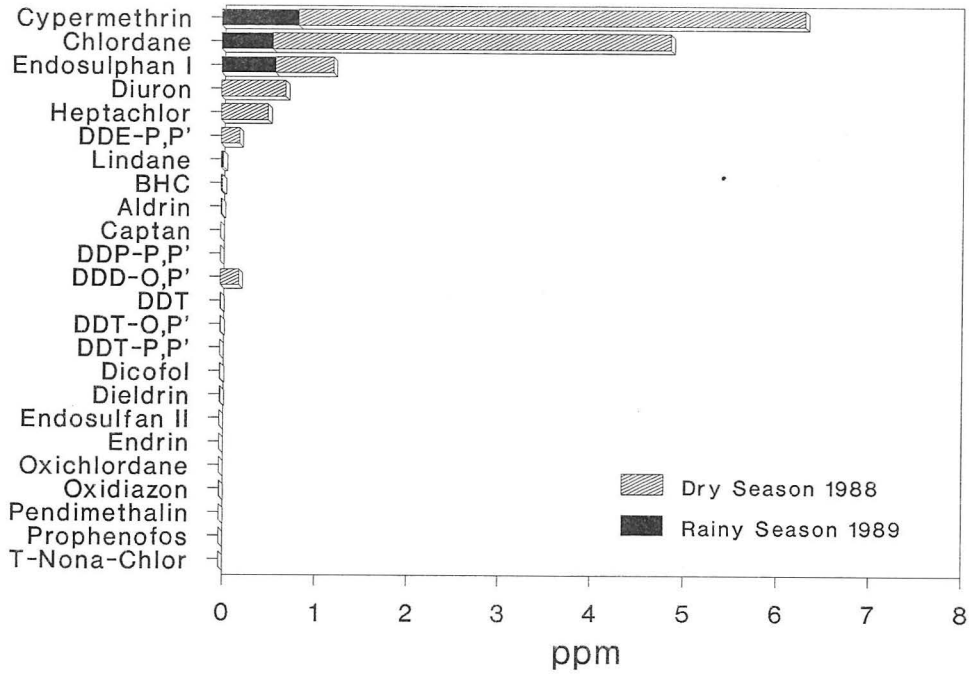

Fig. 5. Mean pesticide residues in cabbage taken from three principal Honduran markets in two seasons (Sánchez et al. 1990).

Bentley (1990), Bentley and Andrews (1990a, b) and Andrews and Bentley (1990) have discussed the pressures that lead to Honduran farmer's heavy reliance on agrochemicals for pest control. Primary factors include a crisis management mindset ("we fetch the pill when we have a headache"), a desire to be modern, and continuous pressure from technical people who either only believe in chemical control or who receive personal benefit when farmers use agrochemicals. Farmers are aware of the health risks they take when they use pesticides and perceive that pesticides induce new and exacerbate existing pest problems. They often explain pesticide treadmill problems as due to the result of adulterated products or the ability of salespersons to seed purchased inputs with pest inoculum.

Scouting and Supervised Control

King and Saunders (1984) stated that preventive insecticide applications are almost always necessary after head formation or when one larva is found in 10 plants. Andrews (1984) provided quidelines for sampling programs and action thresholds for cabbage, broccoli and cauliflower. In cabbage, weekly insecticide applications should be made to seedbeds. After transplant six larvae in 60 plants justifies an insecticide application. Vásquez and Secaira (1990) concluded that visual inspection of cabbage heads is an effective means of making management decisions. They concluded that 50 and 100 samples per homogeneous field provided unequivocal results in 64 and 78% of the cases, respectively; when the population was near the action threshold, sample size to make unequivocal decisions increased to unreasonable levels.

Farmers use various decision criteria to time applications (Ardon and Sánchez 1990c; Carballo and Hruska 1989). Risk-averse farmers and those wishing to minimize managerial time use calendarized sprays. Some farmers apply on the basis of a subjective appraisal of adult DBM population levels, and others evaluate total adult Lepidoptera populations (DBM, pierids and others).

Carballo et al. (1989b) tested in Costa Rica the usefulness of incremental damage levels (i.e. increases in foliar damage from one sampling period to the next) as decision criteria. The 10% new damage threshold provided the highest net income and reduced insecticide applications from 16 to 9 as compared to the calendar treatment.

Bentley and Andrews (1990b) and Andrews and Bentley (1990) questioned the utility of quantitative action thresholds for resource-scarce farmers with diverse economic portfolios. It seems that cabbage in Honduras is a situation in which precise sampling procedures are best considered irrelevant sacred cows of IPM ideologues. They will not be adopted for two reasons. First, they require quantitative sampling and record-keeping skills that farmers do not have, and time that can be better invested elsewhere. Second, the sharecropping arrangement means that all sampling chores fall to the junior partner, while savings accrue to the senior partner. With a strong move toward dependence on microbial insecticides well underway, the Zamorano group has rethought the action threshold concept, turning it on its head; simple scouting procedures should be used by farmers to determine if it is safe to cancel or delay a calendar application.

Cultural Control

Andrews (1984) listed eight cultural practices for DBM. Crop residue destruction has been widely promulgated; major benefits are obtained in management of plant pathogens but the effect on DBM and its parasitoids is not known.

The complex rotational schemes involving six or more crops and cycles of up to 7 years used by Honduran farmers (Ardon and Sánchez 1990a) are probably effective for minimizing soil pest and disease damage, but their role in reduction of DBM damage is not known. There is no basis for evaluating their effect on either DBM or parasitoid numbers.

Honduran farmers often use overhead irrigation systems. Mora (1990) compared DBM infestations in insecticide only plots with irrigated, unsprayed plots and irrigated plots receiving insecticide applications. Lowest DBM incidence and highest yield quality and quantity were obtained when irrigation and insecticides were combined.

Many Central American farmers and some researchers have studied intercrops (Guadamuz et al. 1990). Even in the best cases, intercropping does not appear to provide substantial reduction of DBM populations. The common recommendation to interplant tomato and cabbage is not promising as the former is generally grown at lower altitudes than the latter. Current interest in Honduras focuses on interplanting cabbage and garlic.

Marenco (unpublished data) found so few DBM in the wild plants *Brassica campestris* and *Lepidium* spp. that he concluded they are probably not important alternate hosts. The role of uncultivated host plants in maintenance of natural enemy populations is not known.

Host Plant Resistance

During 1988 and 1989, glossy lines developed by Mike Dickson and collaborators at New York Agricultural Experiment Station were compared in Honduras under farmers' conditions to nonglossy lines from the same source and to commercial hybrids (Mora et al. 1988). Glossy lines suffered much less DBM damage than the others, but at least some seemed to be highly susceptible to *Xanthomonas campestris* (Dowson). When outer leaves were removed, farmers and consumers felt that the glossy lines could meet market standards.

Present and Future IPM Programs for Central American Crucifers

The Zamorano IPM program places emphasis on simultaneous generation and implementation of technologies using methods that stimulate farmer participation and responsive science. While

this approach is not a panacea any more than tech packs or farming systems were (Bentley and Andrews, 1990b), it has led to rapid popularization of implementable technologies. DBM management is being transformeed in Honduras and neighboring countries. However, reliance on microbial control is fragile and needs to be complemented with other procedures mentioned in this paper. Aphids, thrips and pierids become more important when synthetic insecticides are replaced by *B. thuringiensis*. Judicious use of existing tools is needed if more resistance problems are to be avoided. Socioeconomic reality should continue to be the focal point for all IPM work in poor tropical countries. Increasing international trade in the Caribbean Basin, social polarization, economic restructuring, and technological intensification are key processes that are rapidly transforming horticultural production and protection. IPM programs will make use of many technologies for DBM and other pests. Production of pesticide-free produce may be a feasible, lucrative undertaking. The Zamorano program wants to collaborate with other organizations that seek to rationalize pest management practices in crucifers and other crops grown in the tropics.

Acknowledgments

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On-Farm Components of Diamondback Moth Management in Georgia, USA

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Abstract

All too often researchers, farmers and other agricultural consultants overlook the basic principles of on-farm insect management when faced with an insecticide-resistant insect. Although theoretical principles may not always apply when working with farmers, it is important that the researcher/educator becomes the solution to the crisis and not part of the problem. High levels of resistance have been documented in Georgia through the recent National Diamondback Project conducted at Cornell University, New York. Even though very few insecticides currently registered for cabbage or other leafy greens are effective against diamondback moth, *Plutella xylostella* (L.), they can be controlled in cabbage on Georgia farms. On-farm management has become difficult, but we have had continued success using various formulations of *Bacillus thuringiensis* Berliner occasionally tank-mixed with mevinphos, the only organophosphorus compound that continues to perform adequately. We have consistently been able to produce 85% or greater marketable cabbage where these compounds have been used in concert with the following components: use of specially designed high pressure/high volume application equipment; the early, close-interval applications of *B. thuringiensis* with adequate spreader/sticker agents; use of mevinphos only as-needed when diamondback moth populations begin to increase past *B. thuringiensis* sprays; avoidance of the use of insecticides that have proven to antagonize efforts to control diamondback moth. The successful use of these strategies and others have not eliminated the resistance problem, but rather has given us more time to develop other resistance management strategies.

Introduction

The diamondback moth (DBM), *Plutella xylostella* (Lepidoptera:Yponomeutidae), is the single most destructive pest of cabbage and leafy greens in Georgia. Georgia farmers grow 10,000-12,000 ha of turnips, mustard, kale, collards, cabbage and broccoli. In 1989 the economic losses to DBM were about US$16.4 million. Of this, US$2.8 million was spent on cost of control with over US$13.5 million lost in quality and yield (Douce and McPherson 1990). The average farmer in Georgia makes 10 applications of insecticides on DBM-infested crops per growing season. Farmers can produce 2-3 crops/year. Control of DBM on Georgia farms is difficult and many growers lose entire fields and others have poor sales because of quality reductions. However, there are farmers who consistently produce high yields of quality cabbage. The causes of our DBM problems in Georgia and its management principles are discussed below.

Origin of DBM Problem

Georgia's problems begin with the planting of transplants that are often infested with various life stages of DBM. The transplants may have been grown in Georgia or purchased from areas

of the U.S. that are known to have DBM strains with high levels of resistance to insecticides. Also for the last several years, production levels have escalated to meet market demands. This has resulted in growing DBM-attractive crops year-round, thus the DBM problem has been exacerbated through the provision of a continuous high quality food source for DBM and other pests. DBM attacks all types of leafy greens and cole crops during any part of the growing season. The most severe infestations occur from April through October. During this period temperatures range from 21°C at night to 38°C in the day. The potential for 'explosive' population growth is apparent. Since some varieties of cabbage and leafy greens are grown year-round and winter temperatures are mild, DBM populations are present continually.

Insecticide Resistance

High levels of resistance have been documented in Georgia through the recent National Diamondback Project conducted at Cornell University, New York. DBM resistance has been documented for the major insecticide classes: carbamates, organophosphorus and pyrethroids. Populations of DBM from the Tifton area have shown a 180-fold level of resistance to methomyl, a 26-fold level to methamidophos and a 79-fold level to permethrin. And, although very few insecticides currently registered for cabbage or other leafy greens are effective against DBM, they can be controlled in cabbage on Georgia farms.

On-farm Management

On-farm management has become difficult but, we have had good success using various formulations of *Bacillus thuringiensis* Berliner occasionally tank-mixed with mevinphos, the only organophosphorus insecticide that continues to perform adequately. We have consistently been able to produce 85% or greater marketable cabbage where these compounds have been used in concert with the following components: spray delivery systems designed for high pressure/high volume application; early, close-interval applications of *B. thuringiensis* with adequate spreader/sticker agents; use of mevinphos only as-needed when DBM populations begin to increase above marketable thresholds; avoidance of the use of insecticides that have proven to antagonize efforts to control DBM.

Spray coverage of the target is always important for good-to-excellent insect control but, as pests become more tolerant of insecticides, coverage becomes even more important. Sprayers designed to deliver 935 l of water/ha at about 14-28 kg/cm^2 give the greatest practical coverage in our system. Nozzle arrangements vary from sprayer to sprayer but, several drop nozzles arranged at various angles give good results. Broadcast setups perform poorly in general. Delivery speeds should not exceed 6.4 km/hour and boom height should be about 30.5 cm above the crop canopy.

Applications of *B. thuringiensis* should be made every 5 days unless populations are low in the general area. Even then, in Georgia cabbage looper, *Trichoplusia ni* (Hübner), may become the primary pest and frequent applications of *B. thuringiensis* will suppress loopers to an acceptable level. Although field resistance to *B. thuringiensis* in DBM has been shown in other parts of the world (Tabashnik et al. 1990), it has not been documented or observed in Georgia. When DBM infestations develop to five or more per 20 plants, mevinphos is applied for one or two applications. If most of these worms are on the older, nonmarketable foliage, then mevinphos applications may be delayed. Instead, *B. thuringiensis* is applied at either a higher rate or on a closer interval or both. If DBM is the only pest in the field, pyrethroids are avoided because of the antagonism they appear to cause in controlling DBM populations. Pyrethroid sprays are used only when cabbage looper populations exceed threshold levels. Methomyl is also avoided unless beet armyworm, *Spodoptera exigua* (Hübner), develops threshold level populations.

The successful use of these strategies and others is reliant on educational efforts of the extension specialists, researchers, county extension agents, private consultants, agricultural pesticide representatives and farmers. All too often researchers, farmers and other agricultural consultants overlook the basic principles of on-farm insect management when faced with an insecticidally resistant insect. Although theoretical principles may not always apply when working with farmers, it is important that the researcher/educator become the solution to the crisis and not part of the problem.

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Management Approaches for Cruciferous Insect Pests in Central North America

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Abstract

The primary insect pests of cruciferous crops in the midwest United States include root maggots, aphids, thrips and a complex of three lepidopterous larvae. Management of the pest complex has been based primarily on multiple insecticide applications and has resulted in the development of resistance in several pest species, pesticide residues on food, environmental contamination and worker safety problems. In 1987, the diamondback moth, *Plutella xylostella* (L.) caused widespread damage and could not be controlled effectively with conventional broad-spectrum insecticides. Resistance was confirmed in 1988 and 1989 and the most probable source of resistance was identified as resistant larvae imported on transplants grown in southern states. To combat annual importation of varying levels of diamondback moth resistance, a management program was implemented on eight commercial farms in Wisconsin. The program was based on reducing selection pressure on the whole pest complex by treating only at predetermined thresholds, which varied with plant growth stage, and promoting natural control by increasing use of specific bacterial insecticides. In the commercial fields treatment thresholds of 35% infestation with any species of lepidopterous larvae prior to cupping, 25% infestation during cupping and 15% during heading were implemented. An average of 2-4 applications of *Bacillus thuringiensis* Berliner var *kurstaki* for diamondback moth control followed by 1-2 late-season applications of a pyrethroid insecticide for cabbage looper were required and all fields were rated as 95-100% marketable, using fresh market standards, at harvest.

Introduction

Cruciferous crops in the midwestern states of the U.S. are susceptible to damage by a wide range of insect pests. A relatively small complex of key pests occurs annually, however, and management of these pests is essential to meet the stringent marketing standards for damage- and pest-free produce. The key pests are the cabbage maggot *Delia radicum* (L.) (Diptera: Anthomyiiclae), the cabbage aphid *Brevicoryne brassicae* (L.) (Homoptera: Aphididae), thrips *Thrips tabaci* (Lindeman) (Thysanoptera: Thripidae), and a complex of three lepidopterous larvae, the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) (L.), the imported cabbage worm (ICW) *Artogiea rapae* (L.) (Lepidoptera: Pieridae) and the cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae). All key pests are normally present annually and management of the complex has been based primarily on multiple applications of broad-spectrum insecticides. Such unilateral approaches to control have resulted in the repeated development of resistance (McEwen and Chapman 1952; Chapman 1960) in several pest species and subsequent control failures. In response to control failures, growers have habitually switched to newly registered insecticides which have generally provided effective control. In the past decade, however, the availability of new insecticides has become increasingly difficult to predict and, coupled with product withdrawals, cancellations and regulation based on toxicological or environmental standards, it has become important to retain the efficacy of existing materials.

In 1987, widespread control failures occurred with DBM in North America and this insect, which had hitherto been of relatively minor importance in northern growing regions, became the most destructive pest in the lepidopteran complex. Resistance of DBM to registered insecticides which had been widely reported from tropical and subtropical areas of the world (Sun et al. 1986) was found to be widespread in North America in 1988 (Shelton and Wyman 1990). In North America, resistance was most severe in southern states but isolated instances of high resistance were also detected in northern growing regions. The source of resistance in the northern states was shown by Shelton and Wyman (1990) to be primarily through importation of DBM larvae on transplants grown in the south. Shelton and Wyman (1990) also demonstrated close correlation of resistance levels in New York State with resistance in transplant (seedling) production areas in the south.

Since early season production of transplants is not practical in the midwestern states and resistance in the south is endemic, a situation exists whereby midwestern growers must manage highly variable levels of imported resistance annually on a field-by-field basis.

A resistance management program was thus established for Wisconsin growers and implemented in a series of large-scale experimental plots which were established with commercial growers in 1990. The program was designed to reduce selection pressure by crop monitoring and strict adherence to treatment thresholds, and to enhance naturally occurring biological controls by increasing use of specific bacterial insecticides.

Materials and Methods

Eight experimental fields of commercial cabbage varying in size from 0.8 – 4 ha were established in southeastern and central Wisconsin. A resistance management program was implemented in each field (Table 1) to provide cooperating growers with control options that would reduce the potential for DBM resistance and provide effective control of all foliar pests. Fields were scouted weekly by random selection of 25-100 heads which were examined for lepidopterous larval infestation and the presence of other pests. For treatment of lepidopterous larvae, thresholds of 35% infestation prior to the cupping stage, 25% infestation during cupping and 15% infestation during heading, were used. These thresholds were lower than previously published thresholds (Shelton et al. 1983) to permit initiation of DBM control measures at lower population densities and to ensure high levels of marketability based on fresh market standards. A plant was classed as infested when a larva of any of the lepidopterous pests was detected. All species were considered equivalent in this program. Larvae were recorded by size, and pupae were counted but not included in determination of infestation.

Table 1. Field resistance management on cabbage in Wisconsin, 1990.

Pest situation	Recommendation
Early Season (April-June)	
(1) DBM or imported cabbage worm	Use *B. thuringiensis*
Mid Season (July-August)	
(1) DBM or ICW only	Continue *B. thuringiensis*
(2) Cabbage looper present	
a) cupping	Use organophosphorus insecticide
b) heading	Use pyrethroid insecticide
(3) Aphid or thrips present	Use organophosphorus insecticide
Late Season (Sept.-Oct.)	
(1) Any Lepidoptera on heads	Use pyrethroid
(2) Thrips or aphids present	Use organophosphorus insecticide

In the resistance management program (Table 1), the growing season was divided into three time periods, early, mid and late. Since transplanting dates varied between fields from early May to late June, the time of occurrence of pest species relative to the stage of plant growth also varied. In general, however, for a field transplanted in mid May, the early season pests were exclusively DBM in these studies with very few ICW detected. In order to reduce selection pressure from broad-spectrum insecticides, only *B. thuringiensis* (*B. thuringiensis*, MVP or Javelin at 5.2 l/ha) was recommended during early season. Previous studies (Quick 1984) have demonstrated that DBM is heavily parasitized in Wisconsin in untreated plots (83% over 3 years, 1981-84) and that *B. thuringiensis* did not markedly disrupt parasitization levels (57% over 3 years). Early season adherence to *B. thuringiensis* treatments for DBM was thus utilized to promote development of naturally occurring biological control agents. ICW, which can also infest cabbage during early season in Wisconsin and are also susceptible to *B. thuringiensis* treatment, were only detected in these trials at low levels.

In mid season the management program recognized three options depending on the pest situation. If only DBM or ICW were detected the *B. thuringiensis* regime was continued to further promote biological control. If cabbage looper, which is not as effectively controlled by *B. thuringiensis*, was present, a broad-spectrum control was recommended, with an organophosphorus insecticide used during cupping (methamidophos) and a pyrethroid (esfenvalerate or permethrin) used during heading. Other pest species commonly occur during mid season (July-August) and a short persistence organophosphorus insecticide was recommended for thrips or aphid control at this stage to reduce selection pressure on DBM.

During late season, all fields were in the heading stage and could be harvested at any time as dictated by marketing strategies. Pyrethroids were recommended at this stage for all Lepidoptera to provide damage-free produce at harvest. Organophosphorus insecticides were retained for late season thrips or aphid infestations.

At harvest 100 heads/field were rated for damage on a 1-6 scale of increasing damage with 1 representing no feeding on the head or four wrapper leaves, 2 and 3 representing increasing but slight wrapper leaf damage, and 4,5 and 6 representing increasing head damage. All heads with a rating of 3 or below were considered marketable according to fresh market standards.

At the conclusion of the trials adjacent fields, which were treated using the grower's standard program, were compared for insecticidal inputs and marketability.

Results

Although the level of pest infestation varied between the eight trial fields, the pattern of infestation through the season was similar. All fields were infested early with DBM, with infestations increasing to above threshold levels during the precupping period. Two to three applications of *B. thuringiensis* were generally extremely effective in reducing DBM populations to subeconomic levels with MVP and Javelin both providing good control. ICW infestation occurred sporadically and did not reach damaging levels in any fields. *Bacillus thuringiensis* applications were effective in controlling ICW where infestation did occur.

Cabbage looper populations were high in all fields in mid to late season with infestations occurring in late July and August. One to two applications of esfenvalerate provided excellent control.

Aphids were not present in any of the test fields. Thrips did occur in several fields at damaging levels, however, and although parathion was applied, effective control was difficult to achieve.

Damage ratings at harvest were generally excellent with 95-100% marketable produce in all fields. The only loss in marketability resulted from thrip infestation on susceptible varieties.

Survey information is provided for two fields in Tables 2 and 3 to illustrate lepidopteran infestation patterns and efficiency of management actions.

In the Pfeffer field (Table 2) in Racine, Wisconsin, DBM infestation was detected early and by 21 June, the precupping threshold was surpassed and MVP was applied on 23 June.

Table 2. Management of lepidopterous larvae on cabbage, Pfeffer Farm, Racine, Wisconsin, 1990.

Survey date	Heads sampled	% infested	Total lepidopterous larvae/100 head[a]								
			DBM			Cabbage looper			Imported Cabbage worm		
			Small larvae	Large larvae	Pupae	Small larvae	Large larvae	Pupae	Small larvae	Large larvae	Pupae
14 June	100	33	27	10	6	1	0	0	0	0	0
21 June	100	42	31	33	35	1	0	0	0	0	0
27 June	100	6	3	3	34	1	1	0	0	0	0
6 July	100	14	13	2	9	1	1	0	0	0	0
11 July	50	58	60	20	18	0	4	0	10	4	0
18 July	25	4	0	4	24	0	0	0	0	0	0
25 July	100	1	1	0	12	0	0	0	0	0	0
31 July	100	9	0	0	5	13	1	0	0	0	0
7 Aug	50	18	0	0	2	10	12	0	0	2	0
17 Aug	50	0	0	0	0	0	0	0	0	0	0
24 Aug	50	0	0	0	0	0	0	0	0	0	0
31 Aug	50	0	0	0	0	0	0	0	0	0	0

[a] *B. thuringiensis* (MVP 5.2 l/ha) applied 23 June, 15 July; Pyrethroid (esfenvalerate 0.07 kg AI/ha) applied 8 August.

Table 3. Management of lepidopterous larvae on cabbage, Hartung Farm, Arlington, Wisconsin, 1990.

Survey date	Heads sampled	% infested	Total lepidopterous larvae/100 head[a]								
			DBM			Cabbage looper			Imported Cabbage worm		
			Small larvae	Large larvae	Pupae	Small larvae	Large larvae	Pupae	Small larvae	Large larvae	Pupae
2 July	100	19	12	10	8	1	0	0	0	0	0
9 July	100	63	35	53	76	0	2	0	12	12	0
16 July	50	40	18	24	138	1	0	0	0	2	9
23 July	30	17	3	17	53	3	0	0	0	0	0
30 July	33	58	3	9	22	64	9	0	3	0	0
6 Aug	25	60	4	0	4	92	40	0	0	4	0
13 Aug	25	24	0	0	8	12	8	0	8	0	0
23 Aug	25	0	0	0	0	0	0	0	0	0	0
30 Aug	25	12	0	0	0	4	8	0	0	0	0

[a] *B. thuringiensis* 5.2 l/ha applied 12 July, 20 July; Pyrethroid (esfenvalerate 0.07 kg AI/ha) sprayed 30 July, 6 August.

The treatment was highly effective in reducing larval levels but pupae were not affected and a high population of small larvae was produced by the emerging adults necessitating a second MVP spray on 15 July. When plants were in the heading stage, cabbage looper infestation increased to threshold levels and esfenvalerate was applied on 8 August. Control was effective and no further treatments were necessary in this field and 100% marketable heads were produced at harvest. The three-spray regime was as effective as the standard grower practice of 4-5 regular sprays of broad-spectrum materials.

At the Hartung farm in Arlington, extremely high populations of DBM occurred early in the season (Table 3), with 63% infestation on 9 July. MVP was applied on 12 July and although infestation was reduced to 40%, cabbage was in the cupping stage and a second application was made on 20 July which reduced infestation to 17%. Cabbage looper infestation increased suddenly in early August and two applications of esfenvalerate (30 July, 6 August) were required to reduce populations below the heading threshold of 15%. The Arlington field was planted to a kraut cabbage variety with extremely large frame leaves, and insecticidal coverage was poor, accounting for the slow response of looper populations to the pyrethroid applications.

Although relatively large populations of cabbage looper were present during heading, damage was confined largely to frame leaves and 100% marketability was achieved. This level of control was superior to the grower standard practice which utilized two methamidophos applications and two pyrethroids and achieved only 92% marketability.

Discussion

The resistance management program was successful in providing excellent levels of marketability. Early season reliance on *B. thuringiensis* was effective in DBM management in all cases. As plants increase in size and begin cupping, however, pesticide coverage becomes increasingly important. This is particularly evident under dry conditions. The use of only *B. thuringiensis* for DBM control in early to mid season will also promote establishment of DBM parasites which are extremely effective natural mortality factors.

Recent reports of resistance to *B. thuringiensis* in DBM (Tabashnik et al. 1990; Shelton and Wyman 1990) raise concerns regarding the continued effective use of *B. thuringiensis* as a tool to promote natural control and reduce selection pressure from broad-spectrum insecticides. In Shelton and Wyman's survey of *B. thuringiensis* resistance in southern states, however, such resistance was not uniformly distributed among transplant production areas. The importation of *B. thuringiensis* resistance to the north could thus be expected to be sporadic and vary with time and location. Careful field scouting and strict adherence to thresholds to reduce exposure to a minimum would be effective in delaying resistance development to *B. thuringiensis*. The utilization of rapid in-field resistance screening techniques would also help in selecting management options.

The continued use of specific materials such as *B. thuringiensis* is critical in the management of resistance to conventional insecticides, which is present at higher levels and is more widely distributed. The integration of biological control, cultural control, host plant resistance and other management techniques will play an increasingly important role in resistance management for all cruciferous pests in the future.

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Development and Adoption of Integrated Pest Management for Major Pests of Cabbage Using Indian Mustard as a Trap Crop

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Abstract

Use of Indian mustard (*Brassica juncea* (L.) Czern.) as a trap crop for management of diamondback moth, *Plutella xylostella* (L.), and leafwebber, *Crocidolomia binotalis* Zeller, was tried on an experimental farm and farmers' fields. Preliminary studies indicated that planting of 15 cabbage rows followed by paired mustard rows to manage both pests was useful. The first mustard row is sown 15 days prior to planting, and the other is sown 25 days after. During our studies the intercropped cabbage was successfully raised during the rainy season without insecticidal application, however two sprays with 0.05% cartap hydrochloride were necessary to control diamondback moth during winter. Control of insects colonizing mustard was achieved with 0.1% dichlorvos sprays starting from 15 days after sowing at either 10- or 15-day intervals depending on population pressure. Later studies, however, indicated that raising of paired mustard rows at either end of 25 cabbage rows is the most promising planting pattern for successful management of both pests. The intercropped cabbages received two sprays with 0.05% cartap hydrochloride to control diamondback moth and spot application with 0.07% endosulfan and 0.1% phosphamidon to control localized infestation of *Helicoverpa armigera* (Hübner) and aphids, *Brevicoryne brassicae* L. respectively. Mustard also attracted other cabbage pests viz., *Hellula undalis* Zeller and the aphid *B. brassicae*. Several growers have come forward to adopt this technology as a result of the successful on-farm trials and effective publicity.

Introduction

Cabbage (*Brassica oleracea* var. *capitata* L.) is a commercially important cruciferous vegetable and is cultivated in rainy, winter and summer seasons on 10,753 ha in Karnataka State, India (Anon. 1987). Successful cultivation of this crop is hampered due to the incidence of major defoliating caterpillars like the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), and leafwebber, *Crocidolomia binotalis* Zeller (Lepidoptera:Pyralidae). Cabbage webworm, *Hellula undalis* Zeller (Lepidoptera: Pyralidae), tomato fruitworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), and aphids, *Brevicoryne brassicae* L. (Homoptera: Aphididae) also infect the crop in different areas in various seasons (Nagarkatti and Jayanth 1982; Srinivasan and Veeresh 1986). The cabbage growers resort to weekly insecticide applications, and problems arising from widespread use of insecticides are well known. Inadequate control of major pests on cabbage, especially DBM following application of both organophosphorus insecticides and pyrethroids, has recently been reported (Srinivasan 1988). This pest has developed resistance to a wide variety of insecticides in Asia (Chen and Sun 1986; Sun et al.1986; Kao et al. 1989). It is therefore necessary to develop a rational pest management program to reduce the number of applications of hazardous chemicals.

Cultural practices are considered important to suppress pest populations in integrated pest management programs (Brader 1979). Planting of trap crops is one of the cultural methods used for pest management (Metcalf and Luckman 1975). Indian mustard (*Brassica juncea* (L.) Czern) was reported to be a host for DBM and *C. binotalis* (Jayarathnam 1977; Singh and Rawat 1983). Srinivasan and Krishna Moorthy (1991) confirmed the distinct preference for oviposition on mustard by DBM and *C. binotalis* as compared to cabbage. Their field studies showed that a planting pattern of 15 cabbage rows followed by paired mustard rows (first sown 12-15 days prior to cabbage and the other sown 25 days after cabbage planting) was the most promising for successful management of both pests.

We examined the possibility of maximizing the number of cabbage rows that could be raised with mustard without significant reduction in cabbage yield. Further, the results were also verified in two on-farm trials conducted in traditional cabbage areas around Bangalore. The research leading to development and extent of adoption of technology by the growers is discussed later.

Materials and Methods

One field experiment at the experiment station, Hessaraghatta, and two on-farm trials in traditional cabbage areas around Bangalore were conducted utilizing mustard as a trap crop. Ridges and furrows were made in each plot with a spacing of 50 cm between ridges, and 30-day cabbage seedlings were planted 50 cm apart along the sides of ridges. Local mustard (bold seeded type used as an oilseed) was used in all experiments to serve as trap crop. Other details are given in Table 1.

Results and Discussion

Successful use of Indian mustard as a trap crop for management of DBM and *C. binotalis* on cabbage is on record (Srinivasan and Krishna Moorthy 1991). Continuous presence of mustard foliage should be maintained in order to facilitate oviposition by resident and immigrant moths. This is accomplished by taking up an early sowing (15 days prior to cabbage planting) so that mustard attains a height of about 6-8 cm and provides thick and bushy foliage to help attract early arrivals of both pests. Mustard starts flowering around 40 days after sowing and stops producing new foliage. This results in onset of senescence and leaves become unsuitable for larval feeding. In order to maintain continuous foliage, second sowing is recommended in the adjacent ridge on the 40th day after first sowing (i.e. 25 days after cabbage planting).

It is also essential to obtain a reasonable kill of adult moths and larvae sheltered on mustard foliage with a suitable insecticide. Depending on insect pressure, application of 0.1% dichlorvos at either 10- or 15-day intervals is recommended since this chemical has short residual toxicity. It is believed that quick degradation of dichlorvos after every application might have helped the incoming moths to continuously oviposit on mustard without hindrance.

Srinivasan and Krishna Moorthy (1991) also reported that intercropped cabbage could be successfully raised during the rainy season without insecticidal application, whereas two sprays with 0.05% cartap hydrochloride were necessary during winter. The intercropped cabbages received sprays at the time of primordial appearance (initial head formation stage as evidenced by about 2 cm firm leaf ball) since it is a critical crop stage.

Preliminary field studies conducted by Srinivasan and Krishna Moorthy (1991) revealed that 15 cabbage rows can be successfully intercropped with mustard rows to manage both pests. In their study, mustard rows were raised either at the beginning or at the end of cabbage rows.

We felt that the number of cabbage rows could be easily doubled if mustard is raised on either end of cabbage. With this in mind, we increased the number of cabbage rows to 25, 30, 35 and 40 and evaluated them with mustard raised at the beginning and at the end of each plot.

Table 1. Field experiment and on-farm trial details.[a-c]

Particulars	Field experiment	On-farm trials of	
	I	Singapura II	Thalagavara III
Cabbage cultivar	Meenakshi F_1 (Hybrid)	Gloria F_1 (Hybrid)	Express (Inbred)
Season and year	Summer, 1988 (April-Jun.)	Winter, 1988-89 (Nov.-Jan.)	Winter, 1988-89 (Nov.-Jan.)
Plot size	Length: 10 m Width: varied depending on number of C rows	IC: 120 × 100 m C alone: Length 10 m Width: occupying 25 rows of C	IC: 120 × 50 m C alone Length: 10 m and WSC: Width: occupying 25 rows of C
Planting patterns[b]	M rows at the beginning and at the end of 25, 30, 35 and 40 C rows[c] and C alone (25 rows)	IC with M rows at the beginning and after every 25 RC ending in M. 8 IC plots each measuring 120 × 12.5 m C alone (25 rows)	IC with M rows at the beginning and after every 25 RC ending in M. 8 IC plots each measuring 50 × 15 m C alone and WSC (25 rows)
Number and time of M sowing	Two 15 days prior to C planting and 25 days after C planting in the adjacent ridge.	Same as in I	Same as in I
Sprays	IC sprayed with 0.05% cartap hydrochloride at 34, 39 dap. Spot application of 0.07% endosulfan and 0.1% phosphamidon at 53 and 65 dap to control localized infestation of *H. armigera* and *B. brassicae*, respectively.	IC sprayed with 0.05% cartap hydrochloride at 23, 40 dap. Spot application of 0.07% endosulfan at 51 dap to control localized infestation of *H. armigera*	IC sprayed with 0.05% cartap hydrochloride at 30 dap. Spot application of 0.07% endosulfan and 0.1% phosphamidon at 43 and 63 dap to control localized infestation of *H. armigera* and *B. brassicae*, respectively

(Continued)

Table 1. Continued.

Particulars	Field experiment	On-farm trials of	
	I	Singapura II	Thalagavara III
	M sprayed with 0.1% dichlorvos at 15, 29, 39, 49, 69 das.	M sprayed with 0.1% dichlorvos at 26, 37, 55 66, 77 das.	M sprayed with 0.1% dichlorvos 26, 45, 58, 74 das. WSC: Tank mix spray with a carbamate/organophosphorus and pyrethroid at 7-day interval (grower's practice).
Time and nature of observation on C	14, 19, 24, 34, 38, 54 dap for DBM larvae; 34, 38, 54 for *C. binotalis* larvae; 38 dap for *H. undalis* larvae; 54 dap for *B. brassicae.*	21, 39, 50 dap for DBM larvae; 10 and 21, 39, 50, 61 dap for egg mass and larvae of *C. binotalis*, respectively.	10. 29. 42. 58 dap for DBM larvae; 10 and 29, 58 dap for egg mass and larvae of *C. binotalis*, respectively; 58 dap for *B. brassicae.*
Corresponding observation on M	29, 34, 39, 49, 53, 69 dap for respective insect spp.	25, 36, 54, 65, 76 dap for respective insect spp.	25, 44, 57, 73 dap. for respective insect spp.
Sampling unit for C and M	20 plants/plot	10 plants in each plot of IC 20 plants/plot for C alone.	10 plants in each plot of IC 20 plants/plot for C alone and WSC.
Statistical analysis of data (ANOVA, Student 't' and 'Z' statistic -Snedecor and Cochran (1968)	1. Factorial randomized block design for DBM and *C. binotalis* on C using observation time and planting patterns as factors. 2. Mean and SD for *H. undalis* and *B. brassicae* 3. Insect recorded on M in different planting	Nonpaired Student's 't' test for comparison of DBM and *C. binotalis* among M, IC and C alone.	Nonpaired Student's 't' test for comparison of DBM *C. binotalis* and *B. brassicae* among M, IC, C alone and WSC.

(Continued)

Table 1. Concluded.

Particulars	Field experiment	On-farm trials of	
	I	Singapura II	Thalagavara III
	patterns expressed as mean/plant. 4. Conversion of C yield into hectare basis was done depending on area occupied by C in various planting patterns minus cropping under M. 5. 'Z' statistic for comparison of percentage marketable heads.		

[a]C = Cabbage, M = mustard; IC = intercropped cabbage; WSC = weekly sprayed cabbage; SD = standard deviation; dap = days after planting; das = days after sowing (refers to 1 mustard sowing).
[b]C alone and WSC plots (untreated check and grower's practice) was raised at a distance of 200 m from the intercropped block.
[c]First M was sown at 50 cm distance at the beginning and ultimate cabbage row in different treatments. Second M was sown in the adjacent ridge of first M at a distance of 50 cm. Each C plot with M was separated by 20 m fallow.

Adoption of randomized block design for treatment evaluation of this kind may not be always suitable because mustard rows will interfere with insect attraction within and between blocks. Hence we chose to adopt an exploded block design for evaluation. In this layout, each intercropped cabbage plot was separated by 20 m fallow to limit interference in attraction of insects between plots by the mustard foliage.

During summer 1988, the intercropped cabbages received two sprays of 0.05% cartap hydrochloride at 34 and 39 days after planting (dap) (coinciding with primordial appearance) for the control of DBM. Cartap hydrochloride was chosen in view of its effectiveness against DBM as compared to other insecticides including synthetic pyrethroids (Srinivasan and Krishna Moorthy 1988). The mean larval population of DBM recorded among 25 and 30 intercropped cabbage rows was low as compared to the rest of the planting combinations including the control cabbage. The overall reduction in DBM larvae is attributed to combined effects of planting pattern and insecticide application (Table 2). A negligible larval population of *C. binotalis* was recorded in all the intercropped cabbages up to 34 dap. However, plots with 35 and 40 cabbage rows supported 0.4 and 0.5 larva/plant, respectively, as compared to 0.1 larva/plant in 25 and 30 cabbage rows at 38 dap. Increased incidence of *C. binotalis* larvae at 38 dap in 35 and 40 cabbage rows revealed less effectiveness of both planting patterns and application of cartap hydrochloride at 34 dap (Table 3).

We also found that mustard had an added potential to attract *H. undalis* and *B. brassicae*. A larval population of *H. undalis* ranging from 0.6 to 0.9/plant and *B. brassicae* ranging from 13.1 to 18.4/plant was recorded on mustard at 53 and 69 das, respectively, in different planting combinations. Control cabbage recorded 0.8 *H. undalis* larva and 18.3 *B. brassicae*/plant at the corresponding observation days of 38 and 54 dap, respectively. There was no incidence of *B. brassicae* among all the planting patterns of intercropped cabbages up to 54 dap. Low incidence of *H. undalis* (0.1-0.2 larva plant) was observed in 25 and 30 cabbage rows as against 0.7-0.8/plant for 35 and 40 cabbage rows, at 38 dap (Table 3).

Table 2. Population of DBM in different cabbage (figures in parentheses denote corresponding population on mustard).

DAP/DAS	DBM larvae/plant on C and M in				
	25 RC/ MR	30 RC/ MR	35 RC/ MR	40 RC/ MR	C alone
14/29	0.2 (0.3)	0.2 (1.2)	0.3 (1.0)	0.4 (1.2)	1.1
19/34	0.4 (0.5)	0.6 (0.6)	0.9 (0.8)	1.1 (0.8)	1.4
24/39	0.0 (1.2)	0.1 (1.2)	0.4 (1.3)	0.4 (1.4)	1.8
34/49	0.1 (0.7)	0.1 (0.7)	0.1 (0.5)	0.1 (0.5)	1.3
38/53	0.0 (0.0)	0.0 (0.7)	0.0 (0.6)	0.0 (0.5)	1.0
54/69	0.0 (1.1)	0.0 (1.0)	0.0 (0.9)	0.0 (0.7)	1.2
Mean	0.1	0.2	0.3	0.3	1.3

LSD (P = 0.05)	
Observation time	0.16
Planting pattern	0.14
Interaction	0.35

C = Cabbage; M = mustard; RC = rows cabbage; MR = mustard rows; DAP = days after cabbage planting; DAS = days after I mustard sowing

Table 3. Population of *C. binotalis*, *H. undalis* and *B. brassicae* in different cabbages (figures in parentheses denote corresponding population on mustard).

Planting pattern	Insects/plant days after cabbage planting/days after I mustard sowing							
	C. binotalis larvae				*H. undalis* larvae		*B. brassicae*	
	34/59	38/53	54/69	Mean	38/53	SD	54/69	SD
25 RC/MR	0.1 (3.5)	0.1 (1.1)	0.0 (0.4)	0.1	0.1 (0.6)	0.3	0.0 (18.4)	0.0
30 RC/MR	0.0 (5.6)	0.1 (0.6)	0.0 (0.3)	0.0	0.2 (0.0)	0.4	0.0 (15.4)	0.0
35 RC/MR	0.0 (3.6)	0.4 (0.8)	0.0 (0.2)	0.1	0.7 (0.8)	1.0	0.0 (13.1)	0.0
40 RC/MR	0.0 (9.4)	0.5 (1.2)	0.5 (0.5)	0.3	0.0 (0.9)	1.2	0.0 (13.4)	0.0
C alone	2.4	2.1	2.7	2.4	0.8	0.9	18.3	24.5
Mean	0.5	0.6	0.6					

Factorial randomized block design comparison for *C. binotalis* on different DAP.

LSD (P = 0.05)

Observation time	0.33
Planting pattern	0.49
Interaction	0.85

C = Cabbage; M = mustard;
SD = standard deviation;
RC = rows cabbage; MR = mustard rows;
DAP = days after cabbage planting

Spot application of 0.07% endosulfan was undertaken to control localized infestation of early larval instars of *H. armigera* among the intercropped cabbages at 53 dap. In 25, 30, 35 and 40 cabbage rows 2, 3.3, 3.6 and 5% plants were damaged respectively, whereas in control cabbage 3.4% had damage. On average, 3.4% of intercropped cabbage (inclusive of all the treatments) harbored colonies of *B. brassicae* at 65 dap. It was controlled by spot application with 0.1% phosphamidon on that day.

Highest marketable yield of 67.5 t/ha was recorded for intercropped cabbage with 25 rows (Table 4). Based on reduction in insect incidence and marketable yield criteria, a planting pattern consisting of 25 cabbage rows with mustard was selected for testing in two on-farm trials.

At Singapura, the intercropped cabbage was grown on 1.2 ha receiving two sprays with 0.05% cartap hydrochloride at 23 and 40 dap for the control of DBM larvae. Spot application with 0.07% endosulfan was also undertaken to control localized infestation of *H. armigera* larvae which damaged 5.3% in intercropped cabbage at 51 dap. Control cabbage recorded 4.9% damage on that day. Consistently low larval populations of DBM eggs and larvae of *C. binotalis* were recorded on all the observation days among the intercropped cabbage as compared to control cabbage. Intervention of dichlorvos sprays registered a rise and fall of larval populations of both pests on mustard. It supported significantly higher pest population levels as compared to intercropped cabbages on all the observation days (Table 5). All heads were destroyed in the control cabbage while the intercropped cabbage recorded 93.5% marketable heads.

Intercropped cabbage grown on 0.6 ha at Thalagavara was compared with control (untreated check) and weekly sprayed cabbages (grower's practice). Growers have a tendency to mix at least two insecticides belonging to entirely different groups to control DBM. Hence the grower was advised to spray the usual chemicals which he would have chosen in the normal course. The grower had alternated weekly sprays with a mixture of 0.005% cypermethrin (Ripcord 10 EC) + 0.1% carbaryl (Sevin 50 WP) and followed by 0.01% fenvalerate (Sumicidin 20 EC)

Table 4. Marketable yield of cabbage in different management programs (summer 1988).

Number of C rows with M	Marketable heads (%)	'Z' statistic for comparisons of proportions Number of C rows with M				Marketable yield (t/ha)
		30	35	40	C alone	
25	91.3	2.6	4.8	9.8	29.0	67.5
30	86.3		2.3	8.0	28.6	62.3
35	81.5			6.0	27.9	59.6
40	67.8				24.1	49.6
C alone	0.0					0.0

C = Cabbage; M = mustard. 'Z' statistic tabulated value = 2.0 (P = 0.05).

Table 5. Population of DBM and *C. binotalis* recorded in different management programs and its relationship (Singapura).

DAP/DAS	Larvae/plant			Student 't' value[a]	
	M	IC	C alone	M vs IC	IC vs C alone
			DBM		
21/36	1.5	0.9	1.8	4.4**	4.8**
39/54	1.1	0.6	1.9	4.7**	9.0**
50/65	0.7	0.2	2.1	6.3**	11.8**
			C. binotalis		
Egg mass					
10/25	0.5	0.0	0.6	3.2**	6.8**
Larvae					
21/36	0.4	0.0	0.9	6.3**	9.8**
39/54	3.8	0.1	2.5	5.4**	7.1**
50/65	2.8	0.0	2.6	4.3**	10.7**
61/76	1.3	0.0	3.1	7.2**	25.2**

DAP = Days after cabbage planting; DAS = days after I mustard sowing; M = mustard; IC = intercropped cabbage; C = cabbage.
a**Significant at 1% probability level.

+ 0.05% oxydemeton methyl (Metasystox 25 EC). Ten such sprays were applied at 7-day intervals terminating on the 70th dap, before the crop was harvested at 75 dap.

The intercropped cabbage received one spray with 0.05% cartap hydrochloride (coinciding with primordial appearance) for the control of DBM at 30 dap. Localized infestation of *H. armigera* and *B. brassicae* was observed in 6.2 and 3.3% of the plants among intercropped cabbages at 43 and 63 dap, respectively. Spot application of 0.07% endosulfan and 0.1% phosphamidon was undertaken to control *H. armigera* and *B. brassicae*, respectively, in the affected plants. While there was no incidence of *H. armigera* in plots sprayed (weekly), the control cabbage recorded 4.5% damage at 43 dap.

Populations of DBM, *C. binotalis* and *B. brassicae* recorded in different crops are provided (Table 6). Intercropped cabbages supported the least number of DBM larvae on all observation days. Cabbages sprayed weekly recorded the maximum number of DBM larvae/plant from 29

Table 6. Population of DBM and *C. binotalis* recorded in different management programs and its relationship (Thalagavara).

DAP/DAS	Larvæ/plant				Student 't' value			
	M	IC	C alone	WSC	M vs IC	IC vs C alone	IC vs WSC	C vs WSC alone
				DBM				
10/25	0.3	0.1	0.8	0.0	2.1*	3.4**	–	–
29/44	0.8	0.2	1.3	1.4	5.3**	6.8**	8.0**	0.6 NS
42/57	0.8	0.1	1.7	3.5	3.0**	14.4**	20.9**	5.1**
58/73	0.8	0.0	4.1	6.3	5.0**	13.9**	20.7**	3.2**
				C. binotalis				
Egg mass								
10/25	0.2	0.0	0.3	3.7**	5.1**			
Larvae								
29/44	1.3	0.0	1.1	4.9**	4.4**			
58/73	0.6	0.0	0.7	3.0**	8.8**			
				B. brassicae				
58/73	433.5	0.0	730.0	4.2**	5.6**			

DAP = Days after cabbage planting; DAS = days after I mustard sowing; M = mustard; IC = intercropped cabbage; C = cabbage. [a]**Significant at 1% probability level.

dap as compared to the rest of the management programs. There was no incidence of *C. binotalis* and *B. brassicae* among the intercropped and cabbage sprayed weekly up to 58 dap.

The results obtained in our study amply demonstrate that tank mix sprays lead to increased incidence of DBM. Control, weekly sprayed and intercropped cabbage recorded 0, 20 and 93% marketable heads, respectively, at the Thalagavara on-farm trial.

Grower's adoption

Based on successful outcome of on-farm trials, the Indian Institute of Horticulture Research conducted an IPM field day at the grower's field in Singapura on 28 January 1989.

About 250 cabbage growers in and around Bangalore, staff from the Horticulture Department, Government of Karnataka and University of Agricultural Sciences, Bangalore, attended the function. Details of technology in the form of extension literature were distributed to the growers. Wide coverage on the field day was also given by the press.

Based on the success of adoption, the extension education unit of University of Agricultural Sciences, Bangalore, has come forward to disseminate the technology by laying out adaptive trials at each taluka (county) of Bangalore district through its extension guides. Such adaptive trials are presently being conducted in selected grower's fields at Chikballapur, Devanahalli, Doddaballapur and Malur talukas at the time of writing.

We have also received reports from officers of Horticulture Department, Government of Karnataka, employees of pesticide companies and growers that the technology has already spread to several counties where cabbage is grown in Karnataka State. The extent of adoption, however, is not known.

In the neighboring Tamil Nadu State, the Horticulture Department and the Agricultural University have laid out demonstration trials at Ooty (Nilagiris district) and Kanniwadi (Madurai district) where cabbages are grown.

Recently we have found that 5% neem seed kernel extract (NSKE-water extract) spray gives effective control of all pests on cabbage. Our empirical observations in the field also indicate that there is significant increase in the resident natural enemy, *Cotesia plutellae* Kurdjumov,

population wherever NSKE is sprayed. Hence NSKE spray is recommended instead of insecticides on the intercropped cabbages.

Some growers have reported difficulties in taking up first mustard sowing (15 days prior to cabbage planting) since it involves early land preparation and irrigating the mustard rows. These growers, however, had sown mustard simultaneously with cabbage planting and resorted to an additional round of spray during preheading stages on cabbage to control early pest incidence.

Growers often forget to raise the second mustard row at 25 dap, which resulted in additional 2-3 postheading sprays due to increased pest incidence. To overcome the necessity of second sowing, we are looking for a long-duration mustard variety that produces foliage up to 75 das.

It has become increasingly difficult to grow cabbages around Bangalore, utilizing only insecticides since none of the presently available insecticides or combinations give adequate control of DBM. Several growers in Karnataka and neighboring Tamil Nadu states have voluntarily come forward to adopt IPM technology using Indian mustard as a trap crop in less than 2 years after it was demonstrated. This is mainly because of its effectiveness and low input requirement (0.5 kg mustard seeds required/ha costing US$0.5). It is hoped that the technology would spread to cabbage areas in other Indian states within a few years.

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Development and Implementation of the Yellow Sticky Trap for Diamondback Moth Control in Thailand

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Abstract

Yellow, sticky, cylindrical-shaped traps painted with 5% polybutane were highly effective in catching diamondback moth, *Plutella xylostella* (L.), adults in cruciferous crops in 1988-89. The mass trapping of DBM adults by the yellow sticky trap coupled with use of action threshold and *Bacillus thuringiensis* Berliner sprays were studied in cabbage fields at Kanchanaburi Province in Thailand from December 1989 to February 1990. Traps were set up and the number of diamondback moth adults sticking to the traps were recorded daily. The diamondback moth larvae were counted and *B. thuringiensis* at the rate 2.0 kg/ha was sprayed every 3 days if the larvae reached the action threshold level. Three applications were made for the mass trapping field, whereas the conventional control field required five applicatons of *B. thuringiensis* mixed with mevinphos at a rate 0.48 kg ai/ha at weekly intervals. An average 12.97 moths/trap/day were caught. The catch consisted of 55.9% males and 44.1% females. Marketable yield obtained was 24 t/ha and 12.8 t/ha from mass trapping field, and conventional controlled field respectively. These results indicated that the yellow sticky trap could be used partially to control the diamondback moth in cruciferous fields, and could play a prominent role in integrated pest control of the diamondback moth.

Introduction

Since 1972, diamondback moth (DBM), *Plutella xylostella* L. (Lepidoptera:Yponomeutidae), in Thailand has developed resistance to several insecticides. Recently, DBM has shown moderate resistance to even *Bacillus thuringiensis* Berliner. The high resistance was shown to the organophosphorus group: phenthoate, prothiophos, pyraclofos and mevinphos; synthetic pyrethroids group: fenvalerate, cypermethrin, permethrin, cycloprothrin, ethofenprop, and cyhalothrin L; and insect growth regulators group: chlorfluazuron, teflubenzuron, methoprene, and NK-081 (Vattanatangum 1988; Miyata et al. 1988; Rushtapakornchai et al. 1988, 1990). To date there are very few new insecticides, such as abamectin, diafenthiuron and S-71639, that seem to give satisfactory control of DBM (Rushtapakornchai et al. 1990; Katanyukul et al. 1989).

The farmers still believe in pesticide application on a calendar basis, without due regard to pest population and environmental damage. The Thailand Department of Agriculture policy emphasizes the use of integrated pest control. Therefore, an Integrated Pest Control for Secondary Crops in Thailand Project was established in 1984-89. The integrated pest control technology for vegetable crops, especially crucifiers and onion, has been developed and transferred to extension officers and farmers. The development of integrated pest control of DBM involved:

action thresholds, efficient sampling methods which would reduce the use of either chemicals or labor for scouting, physical control including light trap, screen net house, and biological control which included use of *B. thuringiensis* and release of parasitoids.

A yellow sticky vinyl chloride plate trap was reported to catch greater numbers of adult DBM than others such as a light trap and a pheromone trap. The trap could be used for forecasting adult population density in the field (Sivapragasam and Saito 1986; and Saito et al. 1988). The comparison of various trap materials, trap shape and trap height over the plant showed that the most effective trap was cylindrical painted with 5% polybutane in hexane, and the bottom edge of the trap either 10 or 30 cm over the plant. A yellow plastic container or bucket trap was more effective than the yellow plastic trap. Three cylindrical yellow plastic traps/48 m^2 caught the highest (average 106.5) number of adults per trap. The sex ratio of insects caught was 1:0.47, male to female (Saito et al. 1989; Rushtapakornchai et al. 1989). We present here the results of our mass trapping by yellow plastic bucket trap painted with 5% polybutane in hexane in catching DBM adults in cabbage fields, and integration of action threshold and *B. thuringiensis* at Kanchanaburi Province, Thailand.

Materials and Methods

Experimental field

The experiment was carried out in a cabbage field which consisted of mass trapping field, (20 × 40 m), and conventional control field (20 × 20 m) at Thamuang district, Kanchanaburi Province from November 1989 to February 1990.

Trap

The yellow plastic bucket (25 cm diameter, 25 cm high) was used as a trap. Forty traps were placed in the cabbage field and the distance between traps was 4 × 4 m as shown in Fig. 1.

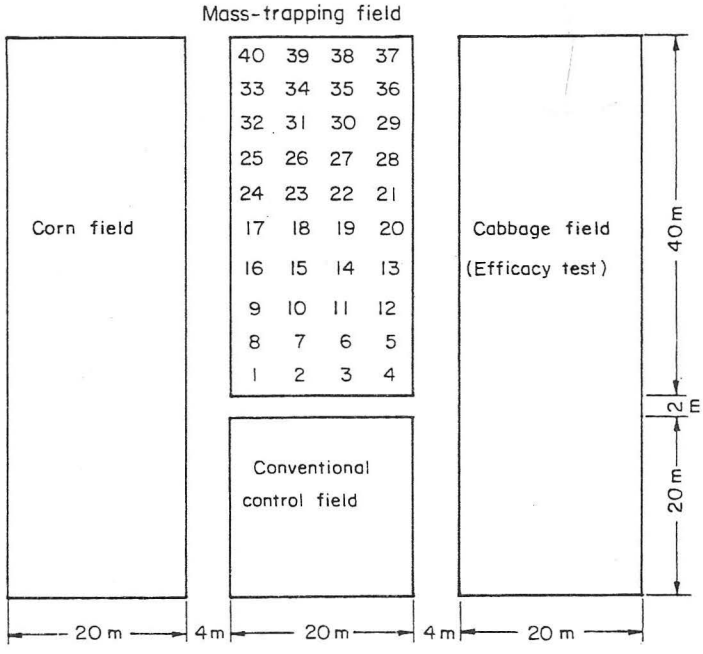

Fig. 1.
Plan of the experimental field; the numbers show the position of traps.

Each trap was nailed to a wooden stake and was placed upright and upside down with the bottom edge of bucket 10 cm above the cabbage plant.

Sticky material

The sticky material was polybutane HT-A (Indemitsu Petrochemical Co., Tokyo) diluted to concentration of 5% in hexane.

Forty traps were placed in the field from 20 December 1989 to 23 January 1990. The traps were painted on the outerside with sticky material once a week. The number of DBM adults caught per trap per day was recorded and the insects removed daily. The number of DBM larvae and the other insect larvae was recorded on cabbage by using a sequential sampling technique for action threshold (Table 1) every 3 days.

Table 1. The action threshold of DBM in a cabbage field.

Sample plan	Threshold level[a]		Sample plans	Threshold level[b]	
	Low	High		Low	High
1-10	10	27	1-5	2	25
1-15	20	41	1-10	20	53
1-20	31	55	1-15	42	82
1-25	42	70	1-20	64	111
1-30	54	84	–	–	–

[a]For 1-35 days after transplanting (before heading period). [b]For over 35 days after transplanting (heading period).
Note: 1 cabbage looper = 20 DBM larvae.

Insecticide application

Mass trapping field was sprayed immediately with *B. thuringiensis* (Delfin WG, Sandoz Co., Ltd.) at a rate of 2.0 kg product per hectare when the number of DBM larvae reached the action threshold. The conventional control field was sprayed with a tank mix of Delfin WG at a rate of 2.0 kg product and mevinphos 24 EC at a rate of 0.48 kg AI/ha at weekly intervals.

Results

Population of DBM adults in yellow traps

A total of 22,829 DBM adults were caught in the 40 traps in mass trapping field (Table 2). There were 12,773 males and 10,056 females, and the average sex ratio was 1:0.79, male to female. The number of males and females trapped and sex ratio were almost the same as those found by Sivapragasam and Saito (1986). Koshihara (1986) and Bhalla and Dubey (1986) reported that the number of males was greater than females caught in all traps and the sex ratio was 1:0.79, 1:0.62 and 1:0.84, males to females. This phenomenon suggested that either the males were more active in searching to mate or they were more responsive to yellow color than females. This might be the effect of the diameter of each facet of eyes in male being larger and the number of facets of male being greater than the female (Wang 1982).

Effect of mass trapping and action threshold

When the mass trapping field was incorporated with action threshold of DBM, the initial threshold was 10-27 larvae per 10 plants. In late growth stage the threshold was 20-53 larvae per 10 plants. The mass trapping field required three applications of *B. thuringiensis* whereas

the conventional controlled field required five applications of mixtures of *B. thuringiensis* and mevinphos (Table 3). The mass trapping field showed that it not only reduced the number of insecticide applications but it also provided a higher yield and less infestation when compared to conventional control field. The number of DBM adults caught in all traps was high, therefore the number of larvae in the field might be high, because a female can lay on average of 80.8 eggs/day (Bhalla and Dubey 1986). The results indicated that the yellow trap can catch DBM adults before oviposition. Generally, the moths are active before dusk and oviposition begins shortly after dusk. The period from pupa emergence to the first peak of oviposition is 32-40 hours, and mating occurred on the day of emergence (Sakanoshita and Yanagita 1972). The egg mortality is 1.8-17.9%, and large numbers of eggs laid are nonfertile if the male to female ratio is 1 (Sivapragasam et al. 1988; Yamada 1979).

Table 2. Number of DBM adults caught by yellow plastic bucket sticky traps in cabbage field.

Days after transplanting	No. adults caught/40 traps		Sex ratio, male to female
	male	female	
24-30	2172	1584	0.72
31-37	939	1113	1.18
38-44	4987	3805	0.76
45-51	2282	1117	0.48
52-58	2413	2437	1.01

Table 3. Sequential sampling of DBM larvae on cabbage in mass-trapping field.

Observation date	No. of DBM larvae/10 plants	
	Mass trapping field	Conventional controlled field
23 Dec. 1989	18 (622)	–
26 Dec. 1989	24 (318)	–
29 Dec. 1989	28[a] (822)[a]	–
1 Jan. 1990	17 (296)	–
4 Jan. 1990	30[a] (790)	–
7 Jan. 1990	8 (2402)	–
10 Jan. 1990	6 (846)	–
13 Jan. 1990	6 (803)	–
16 Jan. 1990	88[a] (379)	184
19 Jan. 1990	32 (195)	156
22 Jan. 1990	31 (615)	159
No. of infested leaves removed/plant	14.2	15.9
Head weight, kg/head	0.60	0.02
Marketable yield, t/ha[b]	24.0 a	12.8 b

[a]Sprayed Delfin WG at 2.0 kg/ha ( ) = number of DBM adults caught on a day before larvae count. [b]LSD at 5% = 9.2; 1% = 13.2.

Discussion

In this study, the adult DBM mass trapping, incorporated with the use of action threshold for control of DBM in cabbage fields, has given promising results. The decline in insecticidal inputs of more than 50% and increase of marketable yield by nearly 100% are sufficient to favor the use of yellow sticky traps over the existing chemical approach practiced by farmers. The greater number of DBM adults trapped per day indicated that yellow sticky traps could be a satisfactory tool for suppression of DBM populations in integrated pest management programs. Research efforts will continue to determine the use of other simplified traps and sticky

materials and finding the optimal number of traps to be used per unit area to refine the integration of these components. In future studies, natural enemies such as egg parasite *Trichogrammatoidea bactrae* Nagaraja, a key parasitoid of DBM in Thailand (Keinmeesuke et al. 1989) will be incorporated as one component in integrated pest management programs in cruciferous crops.

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Management of Diamondback Moth in Malaysia: Development, Implementation and Impact

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Abstract

With the discovery of the larval parasitoid, *Cotesia plutellae* Kurdjumov, and the successful introduction and establishment of two other exotic parasitoid species, viz. *Diadegma semiclausum* Hellen and *Diadromus* (*Thyraeella*) *collaris* Gravenhorst in the Cameron Highlands, biological control was given greater emphasis in the development of IPM for diamondback moth, *Plutella xylostella* (L.), in Malaysia. An IPM package, based on a three-tiered economic threshold level which takes into account the percentage parasitization of the diamondback moth, was developed in 1987. Results of four trials in the highlands and four in the lowlands established the superiority of IPM over prophylactic control practiced by cabbage farmers. Marketable yields were 5-6% higher, and up to 6-fold increase in profits were obtained in IPM plots. The number of insecticide applications was also significantly reduced from 7 to 9 times in the prophylactic plots to a maximum of only three in IPM plots. No insecticide residue was detected in the crop harvested from IPM plots. Beginning with this first IPM package, two variant packages that take into consideration crop phenology have been developed and are under evaluation. Activities to implement and promote area-wide adoption of the IPM package have been organized, such as seminars, workshops, courses, dialogues, joint trials and field days. A national level committee for vegetable IPM has also been set up to oversee the systematic development and implementation of vegetable IPM country-wide. Over the last 3 years, IPM has achieved significant impact at all levels. The Malaysian government has adopted IPM as a national policy for vegetable pest management. The Malaysian Agricultural Chemical Association (MACA) is receptive to and supports IPM. In a recent census, 54% of the farmers surveyed in the Cameron Highlands are already practicing IPM of diamondback moth and 85% are keen to learn more about the IPM approach.

Introduction

Cultivation of vegetables is an important agricultural activity in Malaysia. More than 50 types of vegetables, comprising both tropical and temperate species, are planted (Ding et al. 1981). The important leafy brassica vegetables are Chinese mustard (*Brassica chinensis* var. *oleifera*) and cabbage (*Brassica oleracea* var. *capitata*).

The major insect pests of leafy brassica vegetables in Malaysia are presented in Table 1. The ranking shows that the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), is the most important insect pest. DBM was first recorded in Malaysia in 1925. By 1941, it had become widely established all over Malaysia as a serious pest of brassicas (Corbett and Pagden 1941).

Major interest and research on DBM in Malaysia began in the early 1960s as a result of heavy crop losses due to the pest. Outbreaks and high incidences were reported yearly with

resurgence occurring every 2-3 years (Lim 1974; Sudderuddin and Kok 1978). Since the 1940s, the main method of control practiced by farmers has been the use of synthetic insecticides. The demand for insecticides has been enormous and seems endless, generally accounting for about 30% of the production costs (Lim 1974). Due to over dependence on chemicals, several pesticide-related problems such as resistance development, hazards to nontarget organisms, environmental pollution, poisoning and residues in crops have surfaced and become serious (Lim et al. 1988).

In the 1970s, an ecological approach was adopted to manage the DBM problem. Research was intensified in biology, ecology and control tactics, particularly biological control, in an attempt to develop a more sustainable approach to manage the DBM problem. We present highlights of the development, implementation and impact of an Integrated Pest Management (IPM) program for DBM in Malaysia.

Development Of IPM

The development of an IPM program for DBM in Malaysia comprised several distinct stages. These ranged from initial appraisal of the problem and examination of strategic options, to component identification, development and integration, and eventual evaluation of interim IPM packages at the farm level. The main stages are described below.

Exploring the available control options

Varietal resistance: Although differences in varietal resistance of cabbages to DBM were known (Weires and Chiang 1973), generally, even the 'resistant' varieties were still inadequate for effective management of the moth under normal field conditions. Moreover, a much greater problem was encountered locally. Here, there was little possibility for selecting and developing resistant materials since cabbage flowering could not be readily induced under local field conditions.

Cultural management: Although Raros (1973) had demonstrated that intercropping of cabbages with tomatoes could reduce infestations of DBM on cabbages, this relationship could

Table 1. Relative importance of the main insect pests of leafy brassica vegetables in Malaysia.

Crop/pests	Ranking in importance
Chinese mustard	
Plutella xylostella	1
Phyllotreta sinuata	2
Agrotis ypsilon	3
Spodoptera litura	3
Cabbage	
Plutella xylostella	1
Hellula undalis	2
Crocidolomia binotalis	2
Myzus persicae	2
Spodoptera litura	3
Agrotis ypsilon	3
Kailan (*Brassica alboglabra*)	
Plutella xylostella	1
Phyllotreta sinuata	2
Spodoptera litura	3
Liriomyza spp.	3

Ranking: 1 = Very important 2 = Moderately important 3 = Occasionally important.

not be reproduced consistently. Moreover, this cultural practice could not be readily implemented locally as the choice of crops for cultivation during a particular period is highly variable, being usually governed by festive seasons and market demands (FAMA 1974). For tomatoes and cabbages, their plantings need not necessarily always coincide. Similarly, other cultural methods (e.g. planting trap crops) will require drastic changes in some other long-standing traditional practices, and such a recommendation is usually not easily accepted.

Novel approaches: Newer and more novel approaches, such as hormonal control (Sato 1968), sterile mating (Bonnemaison 1966) and the use of antifeedants (Findlay 1970; Ruscoe 1972), were still largely of an exploratory nature and mainly confined to laboratory situations. As such, their scope for practical employment was limited. However, there is still current interest in some of these novel approaches, and additional ones such as manipulation of host-finding behavior of DBM parasitoids with kairomones are being studied (Loke et al. 1990).

Biological control: Effective suppression of DBM with such an approach has been reported in Australia, Indonesia, New Zealand and South Africa (Muggeridge 1930, 1939; Ullyett 1947; Vos 1953). Encouraged by these experiences, and noting the existing limitations of the other control components, it appeared that use of natural enemies constituted the most promising alternative component available. The biological control approach, therefore, was given priority consideration.

Initial search for parasitoids

In the past no attempt was made to explore the use of natural enemies against DBM in Malaysia because of the belief that no biological control or parasitoids could exist (in the Cameron Highlands) due to the intensive and indiscriminate use of insecticides. Consequently, all DBM control efforts were devoted solely to the chemical approach, further guided by another belief that no cabbage can be produced without frequent and heavy insecticide application (Lim 1982). Therefore, a thorough and careful parasitoid search was deemed necessary. This was made in 1973, intially, at the MARDI Research Station in Tanah Rata. The search uncovered *Cotesia plutellae*, a larval parasitoid (Lim and Ko 1975). However, no other parasitoid was found, either from the eggs or pupae of DBM.

With the discovery of *C. plutellae*, the first belief was shattered, thus paving the way for the acceptance of biological control agents as a possible alternative component to the existing pesticide dependency. It also opened an avenue for subsequent development of the IPM approach. Until the belief was shattered, IPM for DBM could never be accepted even at the conceptual stage in the earlier days.

Focusing research on biological control

Although *C. plutellae* was initially found in Tanah Rata, it was not known whether the parasitoid was also present in areas where crucifers were cultivated under heavy insecticidal inputs. A number of surveys conducted subsequently revealed it to be both common and widespread in the country (Lim and Ko 1975; Ooi and Sudderuddin 1978; Lim 1982).

Except in the lowlands where parasitism averaged only 12.1%, *C. plutellae* occurred in significant abundance in the Cameron Highlands. Over the various subregions surveyed, Kuala Terla had the highest mean parasitism of 48.6% while Kampong Taman Sedia had the least (12.7%). For the remaining subregions, parasitism of 36.5, 34.0, 30.1 and 18.1% was obtained in Kampong Raja, Tringkap, Bertam Valley and Mensum Valley, respectively. In general, it was very encouraging in that the parasitoid could effect an appreciably high level of parasitism even under heavy insecticidal pressure. Although this suggested possible development of field tolerance to pesticides, subsequent bioassay studies could not confirm it (Lim 1982).

With the discovery of *C. plutellae*, intensive studies were mounted on the parasitoid. These encompassed various aspects on its biology and ecology (Ooi 1979a; Lim 1982), including its impact on DBM (Lim et al. 1986). Continued search for natural enemies subsequently revealed two other parasitoids: an incidental pupal parasitoid *Tetrastichus ayyari* and an unidentified chalcid ectoparasitoid of the family Eupelmidae (Ooi and Kelderman 1977; Ooi 1979a,b). Both these were however of little importance and occurred only in negligible numbers.

Introduction of exotic parasitoids

In spite of its usefulness, natural control by *C. plutellae* was inadequate in providing full suppression of DBM (Ooi 1979b; Lim 1982; 1986; Chua and Ooi 1986; and Lim et al. 1986). Attempts were made in 1975-77 to introduce exotic parasitoids to complement *C. plutellae* (Ooi and Lim 1983). Altogether, four parasitoid species were brought in from Australia, India, Indonesia and New Zealand, viz: *Diadegma semiclausum*, *Diadromus* (*Thyraeella*) *collaris*, *Tetrastichus sokolowskii* and *Macromalon orientale*. Of these, only the first two became established in the Cameron Highlands. *M. orientale* failed to breed in the laboratory and was not released. In total, the number of adult *D. semiclausum*, *D. collaris*, and *T. sokolowskii* released between 1976 and 1978 was 1202. In 1982, 21,225 were released (Ooi 1979a; Ooi and Lim 1983).

Assembling the IPM program

With the acceptance that parasitoids can play a significant role, attempts were then made to begin formulating an IPM program. Initially, the IPM approach as opposed to farmers' practices, where frequent and heavy doses of insecticides were applied, consisted essentially of need-based treatment when the tentative economic threshold of 5 larvae per 10 plants was exceeded. Beyond this level, *Bacillus thuringiensis* Berliner was applied. Other synthetic insecticides were used only when the infestation continued to rise beyond 37 larvae/10 plants. In this first IPM program, the impact of natural enemies was not incorporated in decision-making on pesticide treatment, largely because of inadequate ecological data.

From the first six field trials carried out in the early 1970s in both farmers' fields and experimental farms, it was found that several of the cabbage crops managed with the IPM approach did not prove inferior to those where farmers' practices were adopted (Sivapragasam and Lim 1982; Sivapragasam et al. 1985a,b). The IPM fields, on average, were in fact marginally superior in terms of economic returns.

Improvement of the IPM approach

Over the years as additional information became available, the original IPM approach was continually improved through modifications. The main changes concerned improving decision-making with respect to needed action on treatment. Essentially, these involved refining and improving the adopted thresholds, as well as incorporating the role of parasitoids. For example, the initial thresholds of 5 larvae/10 plants and 37 larvae/10 plants were in later trials modified to 15 larvae and 37 larvae per 10 plants, respectively. These still did not incorporate the contributions of biological control agents. However, later on, parasitoids were included. Here, irrespective of the infestation level of DBM, no insecticide was applied when DBM larvae had at least 50% parasitism level. More recently (1987), however, the thresholds were again modified, respectively, to 4 larvae/plant and 7 larvae/plant with parasitism rate of at least 40% as a subparameter (Table 2). When the first threshold is reached only *B. thuringiensis* would be applied. Conventional chemical insecticides are used only when the second threshold of 7 larvae/plant is reached. Results of four trials in the highlands and four in the lowlands clearly established the superiority of IPM over prophylactic control practiced by cabbage farmers.

Table 2. An outline of the interim IPM program for DBM in Malaysia.

These main steps are designed to help achieve the following objectives:

1. Make a decision on whether to spray or not.
2. Manage applied pesticides judiciously.
3. Encourage build-up and enhance action of biological control agents.
4. Encourage adoption of good agricultural practices.

Spray decision

The decision to spray or not to spray is based on:

1. Economic Threshold Level (ETL) of DBM.
2. Level of parasitization by parasitoids.

Sampling of DBM and Natural Enemies

To obtain the necessary data for spray decision-making, weekly sampling is carried out. The procedure is as follows:

1. Counting of DBM larvae on 60 plants/0.1 ha plot using an alternating U-shaped sampling system.
2. Counting of parasitoid cocoons and pupae from 60 plants.
3. Dissection of 60 or available number of 3rd/4th instar DBM larvae for determination of level of parasitization.

Thresholds adopted

1. No spray – if number of DBM larvae < 4/plant
2. No spray – if number is > 4 but < 7 and level of parasitization is $> 40\%$.
3. Spray – Use *B. thuringiensis* if number is > 4 but < 7 and level of parasitization is $< 40\%$. Example: Bactospeine at the rate of 1.14 kg/ha.
4. Spray – Use synthetic insecticides if number is > 7.

Marketable yields were 5-60% higher and up to 6-fold increases in profits were obtained in IPM trial plots. The number of insecticide applications was also significantly reduced from 7 to 9 times in prophylactic plots to a maximum of only three applications in IPM plots (Fig. 1 and 2).

In general, the series of IPM trials conducted between 1987 and 1990 has shown that IPM can provide a higher net revenue when compared to farmers' practice of using chemicals prophylactically without any regard for natural enemies. IPM also required fewer numbers of sprays and yet was able to secure marketable heads. No insecticide residue was detected in the crop harvested from IPM plots.

To date, experimental studies with IPM have thus shown it to be both promising and highly encouraging. Even without considering the intangibles such as reduced general environmental pollution, fewer upsets of existing natural balance and ecological stability, etc., the decline in insecticidal inputs alone is sufficient to favor IPM over the existing chemical approach of farmers. Clearly, over the long term the ecological benefits are likely to prove highly significant.

Implementation Of IPM for DBM

The implementation of IPM by farmers can only be realized through concerted and sustained support of both research and extension. Relevant personnel, particularly those in extension, would need to help farmers overcome their fears and any related negative perceptions, and to guide them to 'think IPM.' Implementation of IPM must therefore have a holistic approach, taking

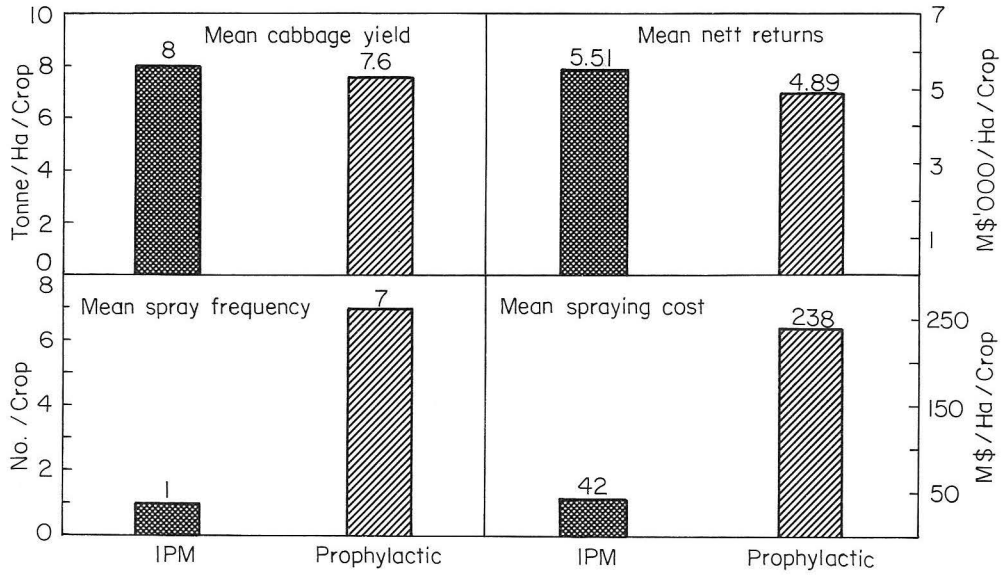

Fig. 1. Summary of IPM trials in the Cameron Highlands.

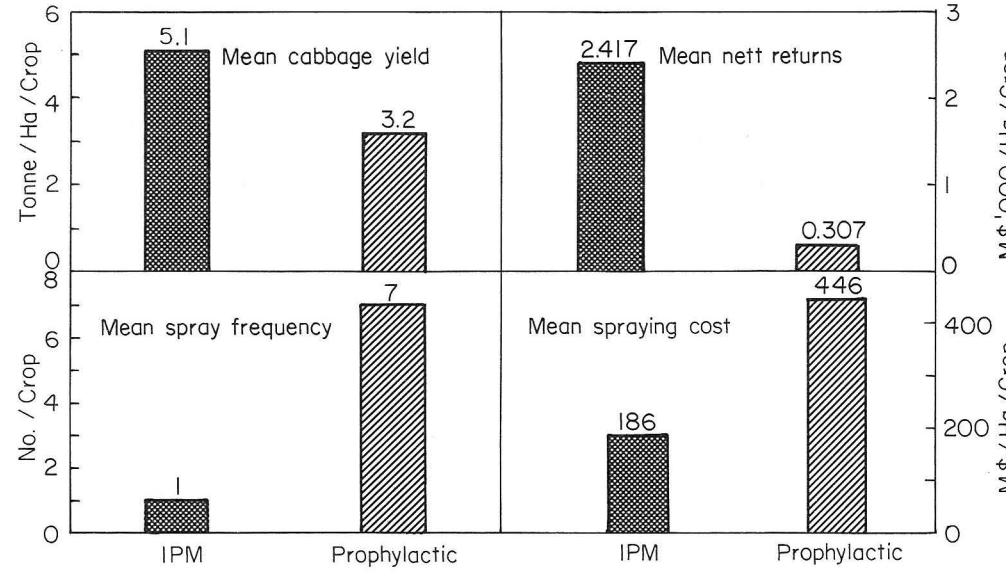

Fig. 2. Summary of IPM trials in Jalan Kebun (lowlands).

into consideration the diverse but related functions of different agricultural agencies, especially the research-extension-farmer linkages which exist in the country.

Extension of IPM for DBM

Presently, this early stage IPM program for DBM is being gradually extended to extension agents and farmers. Some early efforts have involved organizing training courses on IPM for

extension agents, seminars on IPM of DBM, dialogue sessions with farmers and field demonstrations of successful trials. In such sessions, farmers were guided on the role of key beneficial agents and how to recognize them in the field. It is expected that increasing efforts will continue to be expended in this aspect in the future.

Research support

The current IPM technology for DBM is not static. Rather, as it is implemented it will also be modified and improved as new research findings continually become available. For example, two 'new' IPM packages for DBM which take into consideration crop phenology have been formulated and are being evaluated for possible implementation (Table 3). Research support, therefore, constitutes a fundamental aspect in ensuring the constant improvement and continued success of implementation of IPM of DBM.

In general, Malaysian research in DBM IPM is largely borne by the Malaysian Agricultural Research and Development Institute (MARDI). MARDI has a network of 28 research stations spread over the country. At least five of these are deeply involved with research on both highland and lowland vegetables.

Table 3. New IPM packages for DBM.

Crop age	Economic threshold level (ETL)	Decision
1. Current MARDI DBM IPM Package		
Week 1-10	<4 DBM larvae/plant	No spray
	>4<7 and parasitization >40%	No spray
	>4<7 and parasitization < 40%	Spray microbial insecticides (*B. thuringiensis*)
	>7	Spray conventional insecticides
2. New DBM IPM Package (A) under evaluation		
Week 1-4	<4 DBM larvae/plant	No spray
	>4<7 and parasitization > 40%	No spray
	>4<7 and parasitization < 40%	Spray microbial insecticides
	>7	Spray conventional insecticides (*B. thuringiensis*)
Week 5-10	<8 DBM larvae/plant	No spray
	>8<14 and parasitization >40%	No spray
	>8<14 and parasitization < 40%	Spray microbial insecticides (*B. thuringiensis*)
	>14	Spray conventional insecticides
3. New DBM package (B) under evaluation		
Week 1-4	<2 DBM larvae/plant	No spray
	>2<4 and parasitization > 40%	No spray
	>2<4 and parasitization < 40%	Spray microbial insecticides (*B. thuringiensis*)
	>4	Spray conventional insecticides
Week 5-10	<4 DBM larvae/plant	No spray
	>4<7 and parasitization > 40%	No spray
	>4<7 and parasitization < 40%	Spray microbial insecticides (*B. thuringiensis*)
	>7	Spray conventional insecticides

Presently, MARDI has two research divisions (Fundamental Research and Horticulture) which are responsible for research pertaining to production and pests of vegetables. Straddling these divisions is a pool of crop protectionists (entomologists, pathologists, nematologists and weed scientists) who are actively involved in basic and applied research. Under the jurisdiction of these two divisions a task force on IPM of vegetable was specially set up to help develop and implement IPM of DBM.

Local network and external assistance

Local networking is important for effective implementation of IPM. A National Committee for Vegetable IPM with IPM of DBM as its first target, has been set up. MARDI and the Department of Agriculture are the two lead agencies involved in developing, implementing and coordinating the IPM programs nationwide.

External assistance, undoubtedly, could also help greatly in the implementation of IPM of DBM in Malaysia. An excellent example is seen in the case of the FAO Intercountry IPM Program for Rice. Until this network came into effect, implementation and adoption of rice IPM was relatively slow. However, it made great strides when the network provided appropriate training assistance. Undoubtedly for DBM IPM in Malaysia this aspect again constitutes a crucial requirement that could greatly expedite its implementation.

Another important area of assistance which is needed entails receiving expertise for specialized technical aspects. Linkage with AVRDC in this aspect has been fruitful and beneficial.

Apart from the above two broad areas of assistance, others such as material support are not really crucial. This is mainly because much of the material support essential to both research and extension of IPM of DBM is already available in Malaysia.

Impact Of IPM OF DBM

Recognition and acceptance

Over the last three years, IPM of DBM in cruciferous crops has achieved significant impact. The DBM IPM program is now viewed as a guiding model for further development of IPM for other vegetable pests. Due recognition has been received from both public and private sectors. The Malaysian government has adopted IPM as a national policy for vegetable pest management. The Malaysian Agricultural Chemicals Association (MACA) is receptive to and supports IPM of DBM.

Adoption by farmers

A recent survey of some 60 farms in the Cameron Highlands revealed that 54% of the farmers interviewed are already practicing some form of IPM of DBM and 86% are keen to learn more about it. This healthy change in attitude together with a corresponding increase in awareness of the role of natural enemies augur well for the adoption of the IPM approach.

Establishment and build-up of parasitoids

Monitoring of the establishment, conservation and build-up of natural enemies in various crucifer-growing areas such as the Cameron Highlands and Kundasang (Sabah) has shown that parasitoids of DBM are present and doing well.

Increase in crucifer planting

That IPM of DBM has made great strides is also reflected by the fact that the growing of crucifers, particularly cabbages, in the Cameron Highlands has regained momentum with many farmers taking up the crop again (Chay 1990).

Discussion

Developing and implementing a full IPM program for DBM has been a slow process. Many constraints were encountered and these had to be overcome. For instance, research had to be pursued with a holistic perspective. While specialist IPM research needs to be continued and strongly supported, it must also involve close collaboration with the farmers so as to be able to establish those elements of IPM that are likely to be useful in practice (Way 1985).

Another important constraint is the dearth of information concerning the many social and market factors that can influence pest control decisions and actions of the farmers. To date, most research on DBM has centered on biological aspects only. But effective adoption of the IPM technology requires knowledge that extends well beyond this. Thus, to overcome this important constraint, studies must now be initiated to cover the aspects pertaining to better understanding of the vegetable farmer, such as his knowledge, perception, attitude and practices. Arising from these will then be a truer understanding of the farmers' aspirations and constraining factors, which could lead to an improvement in communicative processes for translating IPM for adoption.

To date, crucifer farmers in Malaysia are generally unaware of IPM. Therefore these growers must be guided to 'think IPM'. This may be best achieved through practical demonstrations as well as training which actually involves farmer participation. Here, it needs to be emphasized that training has to be 'on-farm'.

The extension support for vegetables is presently still inadequate. In a survey in Peninsular Malaysia, it was found that only 25% of the vegetable farmers received some extension support services from governmental agencies. An urgent need, therefore, is for increased government support to improve such services for more effective implementation of IPM of DBM.

Presently, there is considerable influence on farmers' control practices through services provided by representatives of agrochemical companies. Usually this is a 'single track' approach of relying only on chemical pesticides. Unless strongly counteracted by increased extension services, this trend of dependency by farmers on pesticide salesmen, and hence pesticides, will remain, thereby slowing down the adoption of IPM of DBM.

Despite the problems and shortcomings addressed above, IPM of DBM in Malaysia has made much headway. A working package is now available, and with further refinements a more pragmatic package, based on a core of biological control, should be ready for area-wide adoption in the very near future.

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Pest management for Head Cabbage Production on Guam

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Abstract

Cruciferous crops on Guam are infested by cabbage webworm, *Hellula undalis* (F.), cabbage cluster caterpillar, *Crocidolomia pavonana* Zeller, mustard aphid, *Lipaphis erysimi* (Davis), armyworm, *Spodoptera litura* (F.), flea hopper, *Halticus tibialis* (Reuter), fire ant, *Solenopsis geminata* (F.), leaf miners, *Liriomyza* spp., diamondback moth, *Plutella xylostella* (L.), corn earworm, *Helicoverpa armigera* (Hübner), and garden looper, *Chrysodeixis chalcites* (Esper.). Because cabbage (*Brassica oleracea* var. *capitata* L.) is one of the economically important vegetables on Guam, we have been developing an integrated pest management program for this crop. *Hellula undalis* is a serious problem on seedlings in nurseries as well as transplanted young plants in the field. Naled was effective in contolling this pest on young seedlings. Radish (*Raphanus sativus* L.) and green mustard (*B. juncea* L.) can be utilized as trap crops in the field for this pest. Similarly, the incidence of *C. pavonana* on cabbage was curtailed by growing Chinese cabbage (*B. pekinensis* (Lour.) Rupr. cv. Tempest), or flowering green mustard next to cabbage. The population of *H. tibialis* on cabbage was also considerably reduced when it was grown with radish, Chinese cabbage or mustard as trap crops. *Liriomyza* spp. population was very low as the introduced parasites effectively suppressed them. *Spodoptera litura* was attracted to cabbage more than other crucifers. Pydrin (fenvalerate) is effective in controlling this pest. *Solenopsis geminata* occasionally became a problem in newly transplanted fields during the dry season. Drenching with diazinon controlled this ant. Diamondback moth was a serious problem in one of the trials conducted in 1988. Seasonal variation in occurrence of this pest was evident and it was noted only in the dry season. Information on diamondback moth in terms of host preference and effective insecticides remains to be determined.

Introduction

Crucifers constitute one of the common groups of vegetables grown in Guam and other Micronesian islands. Main cruciferous crops include cabbage (*Brassica oleracea* var. *capitata* L.), Chinese cabbage (*B. pekinensis* (Lour.) Rupr.), pak choi (*B. chinensis* L.), and radish (*Raphanus sativus* L.). On Guam, a total of 10 arthropod pests have been recognized in association with cruciferous crops: the cabbage webworm, *Hellula undalis* (F.) (Lepidoptera: Pyralidae), cabbage cluster caterpillar, *Crocidolomia pavonana* (F.) (Lepidoptera:Pyralidae), mustard aphid, *Lipaphis erysimi* (Davis) (Homoptera:Aphididae), corn earworm, *Helicoverpa armigera* (Hübner) (Lepidoptera:Noctuidae), armyworm, *Spodoptera litura* (F.) (Lepidoptera:Noctuidae), diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera:Yponomeutidae), garden looper, *Chrysodeixis chalcites* (Esper.) (Lepidoptera:Noctuidae), flea hopper, *Halticus tibialis* (Reuter) (Hemiptera:Miridae), fire ant, *Solenopsis geminata* (F.) (Hymenoptera:Formicidae), and leaf

miners, *Liriomyza* spp. (Diptera:Agromyzidae) (Stevens and Muniappan 1977). *Hellula undalis*, *C. pavonana* and DBM are mostly host-specific to cruciferous crops while the rest have a wider host range.

Hellula undalis is native to the Old World. At present, it has spread to parts of Australia and to the Pacific islands. Distribution of this insect primarily depends on flying ability of the moth (Shirai and Kawamoto 1990). In Taiwan, Chinese cabbage is more heavily infested by *H. undalis* than common cabbage, and the infestation is only seen during the months of June-October (Talekar and Lee 1985). Application of *Bacillus thuringiensis* Berliner has been found not to be effective to control this pest in Singapore (Chang and Pegn 1971) and in Hawaii (Tanada 1956).

Crocidolomia pavonana is a native of Africa and Asia, and is now distributed in tropical regions of Africa, Asia, Australia, and Pacific islands (Waterhouse and Norris 1987). Several studies on chemical control of *C. pavonana* have been documented. Fullerton (1979), for example, reported excellent control of this pest with fenvalerate and cypermethrin in the Cook Islands. In Papua New Guinea, Thistleton (1979) recommended acephate, tetrachlorvinphos and permethrin, and in Malaysia, *B. thuringiensis* was recommended by Ooi (1980).

DBM, a cosmopolitan species of Mediterranean origin, has been studied extensively in many countries. Talekar et al. (1985) compiled information on various aspects of DBM including biology, ecology, sex pheromone, cultural control, biological control, chemical control, insecticide resistance, and integrated control. Recently, trap cropping with mustard was found to reduce the infestation on cabbage in India (Srinivasan and Krishna Moorthy 1988).

In recent years, cultivation of some crucifers on Guam has decreased due to pest problems. Farmers either indiscriminately use available pesticides to overcome the problem or grow crops other than crucifers. Frequent applications of organophosphorus, carbamate or synthetic pyrethroid insecticides have led to the development of insect resistance to chemicals and have also resulted in failure of establishment of parasites or disappearance of the established parasites of DBM such as *Cotesia plutellae* (Kurdj.) (Hymenoptera:Braconidae), *Thyraeella collaris* (Grav.) (Hymenoptera:Ichneumonidae), *Tetrastichus sokolowskii* Kurdj. (Hymenoptera : Eulophidae), and *Diadegma insulare* (Cresson) (Hymenoptera:Ichneumonidae) (Muniappan, R., unpublished data). Although *Cotesia variventris* (Hymenoptera:Braconidae) and *Meteorus* sp. (Hymenoptera: Braconidae), two larval parasites of *H. armigera* and *S. litura*, and *Telenomus* sp., an egg parasite of *S. litura*, were commonly found during 1976-78, they were rare in 1988-90. Obviously, either biological control or application of pesticides alone cannot be adopted as a sole control measure to overcome such a complex pest problem. We, therefore, explored alternative control measures and developd an integrated pest control program for the production of crucifers, especially cabbage on Guam.

Occurrence of Pests on Guam

More than 30 field experiments and surveys of farmers' fields during 1988-90 confirmed occurrence of some pests on cruciferous crops reported by Stevens and Muniappan in 1977. *Hellula undalis* attacked young seedlings of crucifers in the nursery before transplanting and young plants in fields. It caused damage on terminal shoots and midribs of leaves. In severe cases, such damage to apical meristems resulted in the development of multiple heads of cabbage which were small and unmarketable.

Spodoptera litura was another common pest that often attacked cabbage. There was a spatial variation in occurrence of this pest. Similarly, DBM was abundant at certain areas of the island, and also there was a seasonal difference in the incidence of this pest. It was found mostly during the dry season.

During 1988-89, *C. pavonana* was found only occasionally, however in 1990, *C. pavonana* was one of the major pests on crucifers in the fields. *Helicoverpa armigera*, *C. chalcites*, *H. tibialis*, *S. geminata*, *L. erysimi* and *Liriomyza* spp. were of minor importance during this study.

Host Preference

Five trials were conducted at four different locations on Guam to find out host preference of pests on crucifers. All trials were arranged in a randomized complete block design with 3-4 replications and each plot consisted of four rows with a distance of 1 m between rows. The distance between adjacent plants within a row was 0.46 m or less depending on the type of plants. There were eight or more plants in a row and the population of each pest was recorded by counting the number of individuals on sampled plants in the inner rows of the plot. Cabbage and Chinese cabbage were germinated in trays and 3-4-week-old seedlings were transplanted in the fields. All other crops were sown directly in the fields. Prior to planting, all fields were thoroughly prepared by disc plowing and tilling. Then a complete fertilizer of 10-20-20 (N-P_2O_5-K_2O) at 1102 kg/ha was applied and incorporated in the furrows. The fields were irrigated using drip systems. Hand-weeding and application of insecticides and fungicides were done when needed. All data were transformed to square root (X+0.5) before performing analysis of variance and means separation was done by Duncan's multiple range test (SAS Institute 1985).

The first trial was initiated on 12 October 1988 at Dededo in the central-northern part of the island. Tested plants included cabbage cv. KK Cross, Chinese cabbage cv. Tropicana, broccoli (*B. oleracea* var. *italica*), pak choi, rape (*B. napus* cv. Dwarf Essex) and radish cv. Relish Cross. There were three replications. DBM was the major pest in this trial. DBM started to appear on 3 November and the population increased so rapidly during the trial that on 25 November there was an average of 31.6 larvae and pupae/10 plants. Although there was no significant difference ($P = 0.05$) in the number of DBM among the crops tested, radish had the lowest number (17.3/10 plants) and cabbage had the highest number of insects (40.0/10 plants) on 25 November. Other pests found during the trial were *C. chalcites*, *H. undalis*, *S. litura*, *H. tibialis*, *Aulacophora foveicollis* (Lucas) (Coleoptera:Chrysomelidae) (a pest of cucurbits) and *Liriomyza* spp. Because of a low population of these pests, it was not possible to evaluate their host preference.

The second trial was started at Merizo in the southern part of the island on 22 November1988. It consisted of three replications using cabbage cv. C-O Cross, Chinese cabbage cv. Tropicana, turnip (*B. campestris* cv. Just Right), green mustard (*B. juncea*), and Brussels sprouts (*B. oleracea* var. *gemmifera* cv. Jade Cross). The number of insects was counted on 12 and 19 December 1988. The common pests observed in this trial were *S. litura*, *Liriomyza* spp. *H. undalis* and *H. tibialis* and their population on different crucifers is given in Table 1. On 19 December,

Table 1. Incidence of various insects on some crucifers in second trial conducted from 22 November-30 December 1988 at Merizo.

Plant	No. insects/10 plants[a]						
	Spodoptera litura		*Liriomyza* sp.		*Halticus tibialis*		*Hellula undalis*
	12 Dec.	19 Dec.	12 Dec.	19 Dec.	12 Dec.	19 Dec.	12 Dec.
Brassica oleracea var. *capitata* cv C-O Cross	18.7 a	185.7 a	5.9 a	4.3 a	0.3 b	0.0 c	0.7 b
Brassica pekinensis cv Tropicana	0.3 a	27.7 bc	21.0 a	11.3 a	16.3 a	7.7 a	1.7 b
Brassica oleracea var. *gemmifera* cv. Jade Cross	21.3 a	169.0 a	1.0 a	2.0 a	0.0 b	0.0 c	0.0 b
Brassica juncea (green mustard)	1.7 a	40.3 b	3.7 a	4.0 a	5.7 ab	1.3 bc	5.7 a
Brassica campestris cv. Just Right	0.3 a	0.0 a	7.7 a	8.0 a	8.3 ab	2.3 b	2.0 b
Mean	8.5	84.5	7.8	5.9	6.1	2.3	2.0
CV (%)	99.0	34.2	65.4	51.3	53.9	25.8	37.4

[a]Values in each column followed by the same letter are not significantly different at $P = 0.05$, DMRT. Data were transformed to square root (X+0.5) before performing analysis of variance. Original means are shown in the table.

the number of larvae of *S. litura* was very high on cabbage with 185.7/10 plants and Brussels sprouts with 169.0/10 plants. On the other hand, there was no *S. litura* observed on turnip. Chinese cabbage and green mustard had 27.7 and 40.4 caterpillars per 10 plants, respectively. *Liriomyza* spp. seemed to be attracted to Chinese cabbage even though there was no significant difference in host preference of the pest on both 12 and 19 December. *Halticus tibialis* was found more on Chinese cabbage followed by turnip and mustard. Cabbage and Brussels sprouts had very few or no *H. tibialis*. *H. undalis* was observed mostly on green mustard on 12 December.

The third trial was initiated on 28 August 1989 at Yigo field in the northern part of Guam. There were four replications and the crops tested include cabbage cv. K-K Cross, Chinese cabbage cv. Tempest and Saladeer, green mustard, and turnip cv. Tokyo Cross. Data taken on 13 and 18 October 1989 are presented in Table 2. The overall insect population was relatively low although *H. undalis* and *H. tibialis* were major pests. More *H. undalis* larvae were found on green mustard and turnip than cabbage and Chinese cabbage. *Halticus tibialis* was noted on Chinese cabbage cv. Tempest, green mustard, turnip, and less on Chinese cabbage cv. Saladeer, but none was found on cabbage. Larvae of *S. litura* were found mostly on cabbage, but none on green mustard or turnip.

The fourth trial was started on 24 November 1989 with four replications at Yigo. Plants examined were cabbage cv. K-K Cross, Chinese cabbage cv. Tempest, green mustard, turnip cv. Oasis, kohlrabi (*B. oleracea* var. *gongylodes*) cv. Grand Duke , radish cv. Minowase Summer no.3. Data taken on 4 and 9 January 1990 are presented in Table 3. Green mustard, turnip,

Table 2. Incidence of insects on various crucifers observed during third trial conducted from 22 Nov.-30 Dec. 1988 at Yigo.

Plant	No. insects/10 plants[a]				
	Hellula undalis	*Halticus tibialis*		*Spodoptera litura*	
	18 Oct.	13 Oct.	18 Oct.	13 Oct.	18 Oct.
Brassica oleracea var. *capitata* cv. K-K Cross	1.0 b	0.0 b	0.0 c	3.3 a	6.3 a
Brassica pekinensis cv. Tempest	2.5 b	8.5 a	13.5 a	1.0 b	0.0 b
Brassica pekinensis cv. Saladeer	1.0 b	2.8 ab	5.0 b	0.3 bc	3.3 ab
Brassica juncea (green mustard)	15.0 a	6.3 a	11.8 a	0.0 c	0.0 b
Brassica campestris cv. Tokyo Cross	14.3 a	4.3 a	8.8 ab	0.0 c	0.0 b
Mean	5.6	4.4	6.5	0.9	1.6
CV(%)	28.8	36.7	28.9	28.2	55.6

[a]Values in each column followed by the same letter are not significantly different at $P = 0.05$, DMRT. Data were transformed to square root (X + 0.5) prior to performing ANOVA. Original means are shown in the table.

Table 3. Incidence of insect pests on selected crucifers during Trial 4 conducted from 24 Nov. 1989 to 10 Jan. 1990 at Yigo.

Plant	No. insects/10 plant[a]					
	Crocidolomia pavonana		Hellula undalis		Spodoptera litura	
	4 Jan.	9 Jan.[b]	4 Jan.	9 Jan.[b]	4 Jan.	9 Jan.[b]
Brassica oleracea var. *capitata* cv. KK Cross	0.0 b	0.0 b	0.0 b	0.3 b	6.8 a	6.3 a
Brassica pekinensis cv Tempest	64.8 a	110.5 a	0.3 b	0.8 b	0.0 b	0.0 b
Raphanus sativus cv. Minowase Summer Cross No. 3	0.0 b	0.0	5.5 a	28.0 a	0.3 b	0.0 b
Mean	21.6	36.8	1.9	9.7	2.4	2.1
CV (%)	42.8	25.9	32.5	35.1	54.0	50.4

[a]Values in each column followed by the same letter are not significantly different at $P = 0.05$, DMRT. Data were transformed to square root (X + 0.5) prior to performing ANOVA. Original means are shown in the table. [b]There were 6 radish plants observed instead of 10 in replication 4 on Jan. 9.

and kohlrabi were eliminated from analysis of data due to large number of missing plants caused by heavy rainfall resulting in wash-off of seeds. In this trial, *C. pavonana* was found exclusively on Chinese cabbage. Cabbage and radish were free of this pest. *Hellula undalis* was noted more on radish than cabbage and Chinese cabbage. On the other hand, *S. litura* favored cabbage over Chinese cabbage and radish.

The fifth trial was initiated at Inarajan field in the southern part of the island on the same day and with the same six crops as those of the fourth trial. The number of insects was counted on 27 December 1989 and 5, 9 and 18 January 1990. After harvesting turnip and kohlrabi, plots were cleared and replowed, and seeds of radish cv. Minowase Summer No. 3 and South Pole and Chinese cabbage cv. Tempest were germinated on 19 January 1990. Data were taken on 21 February 1990 to determine the difference in host preference at various stages of plant development (Table 4). During the first part of the fifth trial, the highest number of insect pests was observed on 18 January 1990 with the average of 11.3 *Crocidolomia pavonana*, 12.2 *H. undalis* and 7.8 *H. tibialis* on 10 plants. *Crocidolomia pavonana* and *H. undalis* were the two major pests while *Halticus tibialis* was a minor pest. Unlike the Yigo field, there was no *S. litura* observed in the Inarajan field. *C. pavonana* larvae were found on Chinese cabbage whereas cabbage, kohlrabi and turnip had none or very few. Radish had some *C. pavonana*. Observations on 18 January revealed that this pest was attracted to the inflorescence of green mustard. *H. undalis* favored green mustard and radish more than cabbage, Chinese cabbage and kohlrabi. *H. tibialis* was found more on Chinese cabbage, radish, turnip and green mustard. Both head cabbage and kohlrabi had no or a negligible number of *H. tibialis*.

The second part of the trial five included two developmental stages of Chinese cabbage cv. Tempest and radish (January and November planting). Both cabbage and green mustard were at a more mature stage than the first part of the experiment, the former forming larger heads and the latter at the stage of flowering and producing seed pods. Data collected on 21 February 1990 are presented in Table 5. Older plants of Chinese cabbage had highest number of *C. pavonana*, followed by flowering green mustard and young Chinese cabbage plants. No *C. pavonana* were found on head cabbage and radish of both young and mature plants. About 35 *H. undalis*/10 plants were found on older radish of the November planting, while other plants had fewer than 8 larvae/10 plants. Young radish (January planting) attracted most *H. tibialis*. *Lipaphis erysimi* favored mustard plants. Occasionally, *Liriomyza* spp. leaf mines were observed on mustard.

In summary, *H. undalis* was attracted by radish followed by mustard and turnip more than cabbage in the field. It suggests that these crops can be used as trap crops of *H. undalis* in the cabbage fields. Similarly, Chinese cabbage cv. Tempest and flowering mustard can be utilized as trap crops of *C. pavonana*. *Halticus tibialis* was found to prefer Chinese cabbage, mustard, turnip and radish more than head cabbage. *Lipaphis erysimi* favored mustard. Host preference of *C. chalcites*, *H. armigera* and *Liriomyza* spp. was not determined due to low populations of these pests during the study.

Although occurrence of *S. litura* varied from location to location, it favored cabbage more than other cruciferous crops tested. Unlike DBM which appeared in the dry season, S. litura occurred in both dry and wet seasons. Host preference of DBM remains to be determined because it appeared only in the first trial with the extremely high population.

Insecticide Trials

Hellula undalis on cabbage seedlings in the nurseries

Four insecticides were evaluated to control cabbage webworm, *H. undalis*, on young seedlings of cabbage cv. C-O Cross. Seeds were planted in potting media in a 162-cell styrofoam tray on 13 May 1988. Each cell contained 2-4 seeds and eventually plants were thinned leaving one seedling per cell. Five treatments including a check were repeated three times in a complete

Table 4. Incidence of insect pests observed during Trial 5 conducted from 24 Nov. 1989 to 21 Feb. 1990 at Inarajan.

Plant	*Crocidolomia pavonana*			*Hellula undalis*			*Halticus tibialis*		
	5 Jan.	9 Jan.	18 Jan.	9 Jan.	18 Jan.	27 Dec.	5 Jan.	9 Jan.	18 Jan.
Brassica oleracea var *capitata* cv. KK Cross	0.0 b[z]	0.0 c	0.0 c	0.3 b	0.3 c	0.0 c	0.0 c	0.0 b	0.0 b
Brassica pekinensis cv. Tempest	10.0 a	47.5 a	51.5 a	0.3 b	0.0 c	1.3 bc	5.3 b	17.3 a	18.8 a
Brassica juncea (green mustard)	0.0 b	0.0 c	7.0 b	7.0 a	31.1 a	5.5 a	9.8 ab	10.8 a	5.0 ab
Brassica campestris cv. Oasis	1.0 b	0.0 c	0.0 c	3.0 ab	13.5 b	2.3 b	9.0 ab	14.3 a	8.5 ab
Brassica oleracea var. *gongylodes* cv. Grand Duke	0.0 b	0.0 c	0.0 c	0.3 b	0.0 c	0.0 c	0.5 c	0.0 b	0.0 b
Raphanus sativus cv. Minowase Summer No. 3	1.8 b	7.3 b	9.5 b	5.8 a	28.3 a	3.5 ab	12.5 a	19.3 a	14.5 ab
Mean	3.6	9.1	11.3	2.9	12.2	2.1	6.2	7.0	7.8
CV (%)	70.5	52.6	33.8	27.9	45.6	31.9	26.9	36.5	69.9

[z]Values in each column followed by the same letter are not significantly different at $P = 0.05$, DMRT. Data were transformed to square root (X+0.5) prior to performing ANOVA. Original means are shown in the table.

Table 5. Incidence of insect pests on various crucifers observed on 21 Feb. 1990 in Trial 5 at Inarajan.

Plant	No. of insects/10 plants				
	Crocidolomia pavonana	*Hellula undalis*	*Halticus tibialis*	*Lipaphis erysimi*	*Liriomyza* sp.
Brassica oleracea var *capitata* cv. KK Cross	0.0 d[z]	0.3 b	0.0 b	0.0 b	0.0 a
Brassica pekinensis cv. Tempest (Nov. planting)	97.8 a	0.8 b	5.3 b	75.0 b	0.0 a
Brassica pekinensis cv. Tempest (Jan. planting)	10.3 c	7.5 b	10.5 b	0.0 b	0.0 a
Raphanus sativus cv. Minowase Summer No. 3 (Nov. planting)	0.0 d	36.3 a	1.5 b	0.0 b	0.0 a
Raphanus sativus cv. Minowase Summer No. 3 and cv. South Pole (Jan. planting)	0.0 d	6.8 b	37.0 a	0.0 b	0.0 a
Brassica juncea (green mustard) (Flowering)	32.3 b	34.5 a	0.0 b	279.8 a	2.0 a
Mean	30.9	14.4	9.1	59.1	0.3
CV(%)	30.1	34.7	59.2	84.4	43.7

[z]Values in each column followed by the same letter are not significantly different at $P = 0.05$, DMRT. Data were transformed to square root (X+0.5) prior to performing ANOVA. Original means are shown in the table.

randomized block design. Insecticides tested were dipel 2X (0.06 kg AI/264 l), carbaryl 50 WP (0.94 kg AI/264 l), malathion 50 EC (0.8 kg AI/264 l), dibrom 8 EC (0.94 kg AI/264 l). These insecticides were applied by hand-sprayers on 26 and 28 May. The percentage of plants infested by the pest per tray was recorded on 26 (prespray), 27 and 30 May. Data were transformed to arcsine for analysis.

All treatments showed significant reduction in the number of the webworm compared to untreated plants. Dibrom 8 EC was most effective providing 100% control of this pest (Marutani and Muniappan 1988). Application of dibrom seemed to be essential to obtain undamaged healthy seedlings, which were to be planted later in a field, since *H. undalis* is always found to infest seedlings in the nurseries on Guam.

Spodoptera litura on cabbage

Two field trials were conducted to test efficacy of insecticides on *S. litura*, on cabbage cv. K-K Cross. The first trial included fenvalerate 2.4 EC (0.10 kg AI/264 l), dibrom 8 EC (0.94 kg AI/264 l), dipel 2X (0.06 kg AI/264 l), carbaryl 50 WP (0.94 kg AI/264 l), while in the second trial fenvalerate 2.4 EC (0.10 kg AI/260 l), cartap 50 SP (0.09 kg AI/264 l), diazinon AG 500 (0.47 kg AI/264 l) were tested. In the first trial, seedlings of cabbage were transplanted at the Barrigada field in the central part of the island on 29 March 1988. Each plot consisted of five rows with 12 plants/row. The distance between rows and between plants within a row was 1.2 m and 0.46 m, respectively. A randomized complete block design with four replications was used. Treatments were applied with back-pak sprayers on 12 and 27 April, 6 and 16 May. On 20 May, the percentage of plants with the insect was recorded by observing 30 plants/plot. Data were transformed to arcsine for analysis. Fenvalerate treatment had lower numbers of *S. litura* compared to the untreated plants (Yalemar et al. 1988a).

The second trial was conducted at Yigo field in the northern part of Guam. In this trial, the same dimension of the plot and field design of the experiment as above were used except there were four treatments including a check. Plants were treated on 7 May 1990 and the number of *S. litura* was counted on six sampled plants per plot on 7 May (prespray) and 10 May. The average number of *S. litura* found on six plants sampled for each treatment were 39.8 in fenvalerate, 23.0 in diazinon, 23.3 in cartap and 26.3 in check. Three days after spray, the number of *S. litura* on treated plants with three insecticides was much lower than those untreated. Average number of *L. litura* on six plants sampled was 2.3 in fenvalerate, 6.3 in diazinon, 9.3 in cartap, and 39.8 in untreated plots.

Solenopsis geminata on transplanted seedlings of cabbage

Seeds of cabbage cv. C-O Cross were germinated in a nursery tray and transplanted on 1 August 1988 at Barrigada. The size of plot was 2.4 m wide and 3.6 m long and each plot consisted of two rows with 0.46 m space between plants. The experiment was arranged in a randomized complete block design with five treatments including a check and four replications. A drip irrigation system was used since this is the most common irrigation system used by farmers in vegetable production on this island. Insecticide treatments included Vydate L (0.12 kg AI/264 l), methomyl L (0.05 kg AI/246 l), carbaryl 50 W (0.45 kg AI/264 l), and diazinon AG 500 (0.47 kg AI/264 l). They were applied on 3, 8 and 12 August with use of a backpak sprayer. Efficacy was evaluated by counting the number of infested plants by the ant and dead plants due to ant attack on 5, 8, 10, 12, and 15 August. The ant population was relatively high. They made nests under drip lines and girdled the seedlings. Diazinon treatment reduced the ant population more than other treatments (Yalemar et al. 1988b). As a result, there was lower mortality of plants in this treatment.

Biological Control

During 1971-76 attempts were made for biological control of DBM by introducing *C. plutellae*, *T. collaris*, and *T. sokolowskii* from the Commonwealth Institute of Biological Control substation in India and *D. insulare* from the Hawaii Department of Agriculture. Of these introductions, only *C. plutellae* established in the field for a short period and then disappeared. A low percentage of *Chelonus blackburni* Cameron (Hymenoptera:Braconidae) has been observed on DBM since 1976. The failure of establishment of parasites of DBM on Guam is primarily due to frequent use of pesticides in the field for the control of other associated cruciferous crop pests.

Cotesia variventris and *Meteorus* sp. have been observed on the larvae of *H. armigera* and *S. litura* in 1976-78. However, these parasites became rare during 1988-90. Similarly, *Telenomus* sp., an egg parasite of *S. litura*, was very common in 1976-78, but its incidence was low in cruciferous crop fields during 1988-90. A larval parasite, *Copidosoma* sp. (Hymenoptera : Encyrtidae) occurs rarely on *C. chalcitens* in Guam.

Integrated Pest Management

Hellula undalis is the common pest of seedlings of head cabbage in the nurseries. It can be effectively controlled with the insecticide naled. The incidence of *H. undalis* on cabbage in the fields can be reduced considerably by growing radish as a trap crop. *Solenopsis geminata* is a problem in the newly transplanted crops in certain areas of Guam during the dry season. Drenching with diazinon was effective in controlling this ant. *Halticus tibialis* and *C. pavonana* are attracted to Chinese cabbage cv. Tempest. Using it as a trap crop, cabbage can be protected from these two pests. *Spodoptera litura* prefers cabbage over other crucifers tested. The insecticide, fenvalerate, controls this pest. Incidence of *H. erysimi* on cabbage can be reduced by growing green mustard as a trap crop. The population of *Liriomyza* spp. has been suppressed by introduction of exotic parasites into Guam. The incidence of *H. armigera* and *C. chalcitens* was low during the period of our trials. DBM occurred only once during the dry season in the last 3 years. As a result, we could not confirm the results of Srinivasan and Krishna Moorthy (1988) planting mustard as a trap crop for control of DBM in cabbage fields.

We plan to further integrate use of trap crops, insectides and natural enemies of pests of cruciferous crops and eventually develop a program that is safe, economical and effective for control of cruciferous crop pests on Guam.

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Crucifer Seed Crop Pests, Parasites, and the Potential for IPM in Northern Thailand

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Abstract

Seed production of *brassica* vegetables has expanded rapidly in northern and northeastern Thailand during 1988-90, and simplified IPM techniques are urgently needed. A quick and simple sampling procedure for Chinese kale (*Brassica oleracea* var. *alboglabra* Bailey) seed crops was developed based on the close association found between the number of diamondback moth, *Plutella xylostella* (L.), larvae per plant and the percentage of plants infested. Insects were counted twice weekly in the Chinese kale seed crop in 1989-90. Major pests were diamondback moth and armyworm (*Spodoptera litura* F.); minor pests included fleabeetles (*Phyllotreta sinuata* Steph.), aphids, cabbage looper (*Trichoplusia ni* Hübner), *Helicoverpa armigera* Hübner, and cabbage webworm (*Hellula undalis* F.). Diamondback moth populations peaked in January but declined rapidly thereafter; this decline coincided with increasing crop maturity and 45-50% *Cotesia plutellae* Kurdj. parasitism. *Spodoptera litura* populations peaked in early December but also declined rapidly; low numbers of *S. litura* coincided with increased parasitism by *Snellenius* (= *Microplitis*) ? *manilae* Ashmead. Rearing of field-collected diamondback moth larvae and pupae from Chiang Mai and northeast Thailand revealed a surprising diversity of parasites including *Cotesia plutellae*, *Diadromus collaris* Grav., *Macromalon orientale* Kerr. , *Isotima* sp., *Brachymeria excarinata* Gahan, and *B. lasus* Walker.

Simplified IPM for Diamondback Moth in Crucifer Seed Crops

Commercial production of crucifer seed began only recently in Thailand but is rapidly expanding. Several hundred small farmers in the northeast grew *Brassica* seed crops on more than 50 ha during the 1989-90 dry season; more than 75% of this area was planted in Chinese kale (*Brassica oleracea* var. *alboglabra* Bailey). The quantity of Chinese kale seed imported is still higher than for any other vegetable crop, ranging from 120 to 150 t annually with a value of US$240,000-320,000. Local production has supplied less than 25% of this amount leaving ample room for further expansion.

Crucifer seed production is problematic in a tropical climate where temperatures can reach 40°C during the cool season and where insects like the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), can attain as many as 25 generations per year. Unfortunately, DBM populations reach their highest levels during the winter growing season. The long season is both an advantage and disadvantage in terms of pest management. The crop must be protected over a long period with heavy expenditures for pesticides in outbreak years. On the other hand, there is ample time for buildup of beneficial insect populations if growers are careful in choosing insecticides not detrimental to parasites and predators. Fortunately there is no concern over the cosmetic appearance of the product.

Although bilateral and multilateral aid agencies have promoted and supported IPM projects in developing countries for some years now, it is difficult to cite examples of sustained and

effective pest management programs in Asia. In one of the rare cases where researchers returned to evaluate adoption of an intensely promoted IPM scheme, it was found that farmers no longer monitored insects in their fields in spite of all the training they had received--counting insects was just too difficult and time-consuming (Adalla et al. 1989). Most farmers and researchers in Western countries would agree and hire professional scouts or graduate students to do their counting.

Many have observed that IPM technologies used in developed countries cannot be 'transferred' with any hope of success unless they are first simplified and adapted to local constraints (Goodell 1984; O'Neil et al. 1989; Smith 1983). Given the dearth of IPM technicians in the field (especially for horticultural crops), it looks as if most small farmers in Asia will need to do their own sampling and make their own decisions for the forseeable future. They are not likely to use any complex sampling plans or economic thresholds offered them by researchers without drastic simplification.

Chinese kale seed fields were planted at Maejo with the following objectives in mind: (1) to test an IPM scheme using simple action thresholds based on feeding damage in conjunction with selective insecticides (prior to testing in farmers' fields); (2) to verify and document what we considered to be major and minor insect pests at different growth stages of a *Brassica* seed crop; and (3) to try out a standard terminology for describing growth stages for Chinese kale seed production.

Materials and Methods

General

Insect pests were sampled from a Chinese kale seed production field planted at the Maejo Institute of Agricultural Technology, 10 km north of Chiang Mai, Thailand. Maejo lies in the Chiang Mai valley 700 km north of Bangkok at an elevation of 300 m. We sampled from a 440 m^2 field that had been planted as part of a study of insect pollinators of Chinese kale and which included open and caged plots in a randomized complete block arrangement. There were no untreated or conventionally treated check plots for statistical comparisons with plots treated according to thresholds; however, a 230 m^2 plot planted adjacent to the IPM field was sprayed weekly and used for yield and economic comparisons with the experimental field.

An open-pollinated Chinese kale selection from the East West Seed Company was transplanted on 30 October 1989 at 5 weeks after seeding. Seedlings were transplanted into raised beds 5 m long and 1.5 m wide with 50 cm between double rows and 40 cm between plants; other cultural practices currently recommended for commercial *Brassica* seed production in Thailand were followed.

Sampling

We first attempted sampling lepidopterous larvae by counting new feeding holes/plant as this appeared to be one of the easiest and quickest methods available (Chalfant et al. 1979; Workman et al. 1980; Chelliah and Srinivasan 1986). This approach was soon abandoned, however, since it was difficult to separate new and old damage and because clusters of *Spodoptera litura* F. (Lepidoptera: Noctuidae) larvae made so many feeding holes as to render the counts meaningless. Other workers have experienced similar problems with this technique (Cartwright et al. 1987).

Sampling based on the percent of plants infested seemed promising in terms of time and simplicity (Morisak et al. 1984), but must be based on a previously established relationship between the number of larvae per plant and the percentage of plants infested (Kirby and Slosser 1981). We consequently decided to count all larvae during the 1989-90 dry season and to record the number of plants infested with one or more DBM larvae in order to determine this relationship.

These data were used to formulate simpler DBM thresholds based on the percentage of plants infested.

All insects were counted on five plants in a row from open plots in each of the four blocks of the experimental field. Every other plant in a row was sampled. Averages from all blocks (n = 20 plants) were used to compare with thresholds and in statistical analyses. Plots were sampled twice weekly beginning on 9 November at 10 days after transplanting and continuing until 8 February (2 weeks before harvest) for a total of 24 samples. Aphid numbers were not recorded, but a plant was counted as infested if more than 20 individuals were observed. The obvious presence of parasites or predators was also noted.

Growth stages

Both crop susceptibility and insect populations vary considerably during the long growing season for seed crops. The crop was divided into the following four growth stages: (1) seedling--from seeding until transplanting at 6-8 true leaves; (2) preflowering--from transplanting until bolting; (3) flowering/pod setting--from the time first flowers appear until most plants have finished flowering; most seed pods (siliques) will have set during this period, and (4) seed/pod maturity--from the end of flowering until harvest.

Action thresholds

After abandoning the feeding damage approach, we used action thresholds based on number of larvae per plant. These thresholds were best guesses based on experience with seed production here and on tests of thresholds in Thailand and elsewhere. Apparently none have been used or published for *Brassica* seed production in Asia, and those we used for the early crop stages were adapted from trials with fresh market cabbage (Rushtapakornchai and Vattanatangum 1982). The thresholds we tried were as follows:

Growth stage	Flea beetles	DBM	Cabbage looper	Aphids
Seedling	1/plant	–	–	40% plants infested
Preflowering	1/plant	3/plant	0.3/plant	40% plants infested
Flowering/podsetting	–	2/plant	–	40% plants infested

Insecticide treatments

Broad-spectrum products are widely used in Thailand with the frequent destruction of pollinators and natural enemies. Insecticides used in this study were chosen for both their effectiveness and selectivity; products toxic to bees could not be used during the flowering period. *Bacillus thuringiensis* Berliner (Florbac FC, 8500 IU/mg) was used exclusively for control of DBM and cabbage looper (*Trichoplusia ni* Hübner, Lepidoptera: Noctuidae). Pirimicarb (Pirimor) was used for aphid suppression and mevinphos (Phosdrin 24EC) for control of *Hellula undalis* Fabricius (Lepidoptera: Pyralidae). Although mevinphos is a broad-spectrum product with high mammalian toxicity, its systemic action and short residual make it an attractive choice against species which feed within plant tissues. *Bacillus thuringiensis* remains effective against DBM in the north at moderate rates of 1.0-1.5 kg/ha in contrast to the apparent tolerance to this material observed in central Thailand. We chose trichlorfon (Dipterex) for use against *S. litura*, although treatment was not necessary during the study. When the characteristic feeding damage of *Spodoptera* was encountered during sampling, we inspected the entire field and removed by hand any new larval clusters and egg masses. The granular systemics carbofuran (Furadan 3G) or isazophos (Miral 2.5G) were recommended for control of fleabeetles (*Phyllotreta sinuata* Stephens (Coleoptera: Chrysomelidae)) in the seedbed before transplanting, but treatments were also not necessary for this insect.

Results and Discussion

Growth stages

The growth stages described earlier were important in determining when different thresholds would be applied and in deciding what plant parts were to be sampled. In actual sampling practice, however, only the preflowering and flowering/pod setting stages were really necessary. Plants in the seedbed (seedling stage) will probably be treated as needed and not according to any thresholds; it should not be necessary to sample or treat during the seed/pod maturity stage in most years.

Pest management vs. weekly sprays

The field treated according to thresholds was sprayed only four times compared to 10 in the adjacent observation plot which was treated weekly up until 2 weeks before harvest. Insecticide costs for the IPM field were US$98/ha (392 Baht/rai) or about one-third those of the field treated weekly (US$271/ha or 1084 Baht/rai). The six additional weekly sprays were all of *B. thuringiensis* plus a sticker; sprays applied later in the season were more costly and time-consuming because of the much larger plant surfaces covered. For example, 1.3 l/ha of *B. thuringiensis* product was applied at 9-24 days after transplanting compared with 2.5 l/ha applied at full flowering (66-73 days after transplanting).

Seed yields were measured from 3m × 5m, four-row plots from each block of the IPM field and from two plots of the same size in the adjacent field treated weekly. Yields from the fourth block were omitted because of the poor drainage and plant growth in that section of the field. The mean seed yield from three blocks of the IPM field was 870 kg/ha (139 kg/rai) compared with 946 kg/ha (151 kg/rai) in the plots treated weekly. Not counting labor costs and using a seed buy-back price of US$3.00/kg, returns after insecticide costs were US$2511/ha (10,044 Baht/rai) for the IPM plots compared to US$2567/ha (10,268 Baht/rai) for the weekly-treated plots. The field treated according to action thresholds showed only slightly more insect damage than the field treated weekly; higher yields from the latter were due in part to better drainage conditions and more vigorous plant growth compared to the IPM plots.

Action thresholds and sampling

It appears that the threshold for DBM of 2 larvae/plant during the flowering/pod-setting stage might be too high and that 1-1.5 larvae/plant would be safer, especially during the critical early flowering period. A higher threshold of 3.5, 4 or even higher is possible toward the end of flowering. Treatment for DBM and other pests should not be necessary after flowering in most years; populations of *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae) should be high during this period if broad-spectrum insecticides were avoided earlier in the season.

The threshold of 0.3 larvae/plant for cabbage looper is probably acceptable during the early preflowering stage until bolting (about 35 days after transplanting) when a higher threshold of 0.6-1/plant might be more appropriate. Loopers appear to cause little damage after bolting; their numbers may have been held in check by parasites and predators.

The percentage of plants infested with one or more DBM larvae was closely associated ($r^2 = 0.97$) with the average number of larvae/plant (Fig. 1). New thresholds based on the percentage of plants infested can be calculated using the quadratic curve fitted to these data within the range of x = 0 - 3.2 larvae/plant. Using the resulting equation $y = 0.02 + 0.568x - 0.097x^2$ (y = % of plants infested and x = larvae/plant), count thresholds 1, 1.5, 2, and 3 larvae/plant are equivalent to 50, 65, 77, and 85% of plants with one or more DBM larvae, respectively. Although a sequential sampling plan based on these data would be desirable from a statistical standpoint, this technique is probably too complicated for use by the majority of growers. Given the fact that most fields here are smaller than 0.2 ha, it should be possible to use percentage

thresholds in a quick and simple sampling procedure based on casual inspection of a standardized sample of 10 plants for the presence/absence of DBM larvae. The counting obstacle is eliminated and the grower knows immediately when a threshold has been reached (without calculations). Simple boards with movable pegs or slides on a scale painted in contrasting colors above and below thresholds could also help growers keep track of sampling.

We hope that this simplified sampling method for DBM can be tested in the fields of a few commercial seed growers; we believe there is a good opportunity for private seed companies, using their own trained extension staff and input supply, to promote pest management and the use of selective insecticides among their contract growers in north and northeast Thailand.

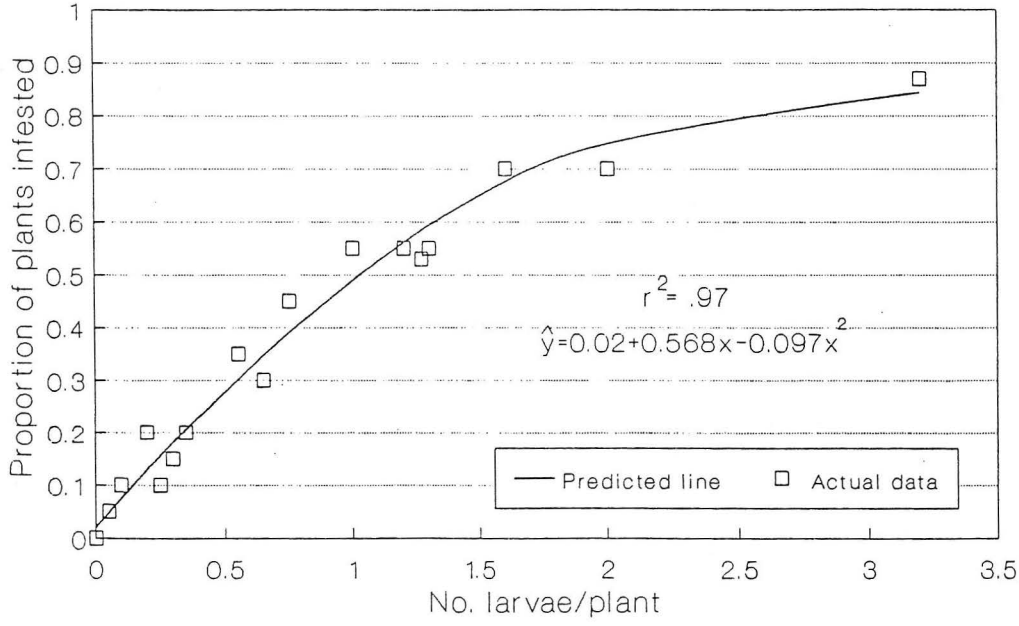

Fig. 1. The relationship between the proportion of infested Chinese kale plants and the mean number of DBM larvae per plant. Data points are means of four replications.

Major and Minor Crucifer Seed Crop Pests

Major Pests

DBM

DBM is considered the most serious pest by both *Brassica* seed and fresh market growers in north and northeast Thailand. Although easier to control in the north than in the central region, DBM is still the major pest. Infestations vary considerably from year to year and even from field to field. In some years the insect is difficult to find and treatments unnecessary while in other years weekly or even more frequent sprays are not able to control the pest. Populations are generally lowest following the June-October rainy season but build up rapidly in December and January. The most critical period for seed producers is the time from about the first week of December until the end of January. Local seed growers have learned that early planting is essential both to ensure flowering during the coolest months and to avoid major DBM damage to seedlings and young plants in the field. Assuming a grower has transplanted on time (mid October to early November), DBM will begin to become a problem during the early to mid-flowering period. At this time flowers and newly formed seed pods must be protected.

Our data from the seed field at Maejo illustrate the scenario just described (Fig. 2). The average number of larvae per plant was low from seeding in late September until bolting at about 45 days after transplanting. The population peaked at almost 3.5 larvae/plant in January during the flowering period but declined without treatment after the cessation of flowering. The rapid decline in the DBM population in February was probably associated with the increasing maturity of the seed crop and with *C. plutellae* parasitism; 45-50% parasitism was recorded from laboratory-reared DBM larvae collected from other crucifer fields at Maejo in February (parasitism by *C. plutellae* and other species reared from DBM at Maejo is discussed later).

Of the four insecticide treatments applied during the 5-month growing season (arrows in Fig. 2), only two of these were applied specifically for DBM. The first treatment (*B. thuringiensis*) on 23 November was made for control of cabbage looper, although most of these larvae were observed outside the plots sampled. The second treatment a week later (mevinphos) was for *H. undalis* larvae, several of which were feeding on flower primordia in the sampled plots. The following two treatments (*B. thuringiensis* + pirimicarb) on 21 December and 4 January were applied for DBM larvae observed on flowers during the critical early and mid-flowering period. The population had reached levels of 1.5 and 1.6 larvae/plant flower cluster for these dates, respectively, but had not yet exceeded the threshold which had been set at 2 larvae/flower cluster. Flower clusters infested with aphids had exceeded the threshold of 40% on both dates.

We made a decision not to treat for DBM in spite of high larval counts after 4 January because flowering had almost ceased and many cocoons of *C. plutellae* were observed in the field. DBM larvae were rarely observed after early February when seed pods were maturing. We had also counted DBM larvae in plots that had been caged with nylon netting for a pollination experiment; DBM populations within the caged plots were very high (>100 larvae/plant) when the cages were removed on 25 January. This was at a time when very few DBM were to be found in the open-field plots. The plots had been caged on 18 December at 50% flowering and had received the same insecticide treatments as the open plots.

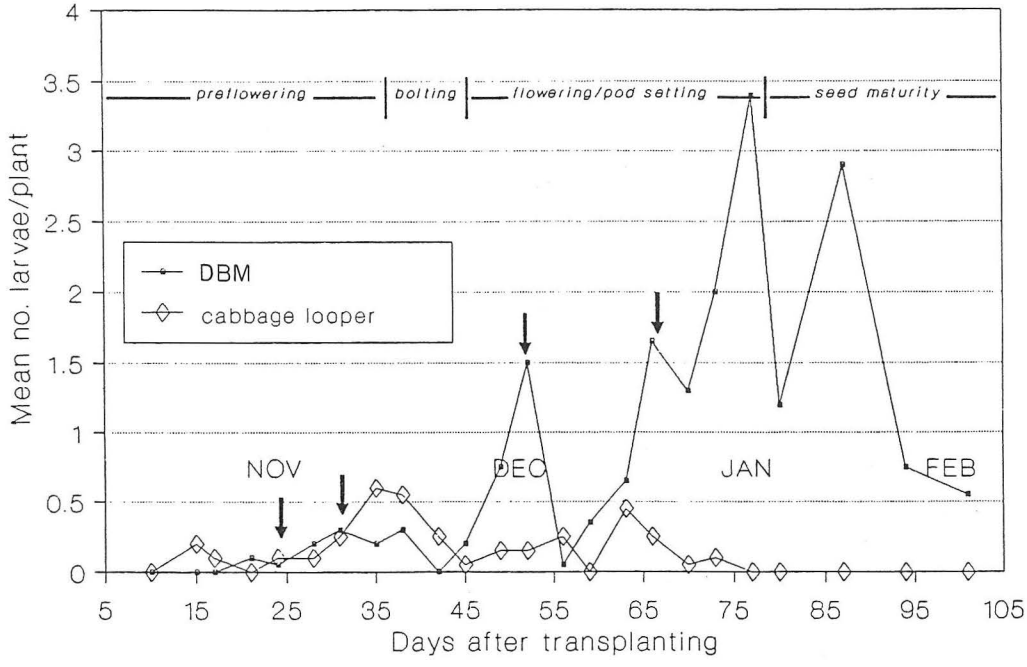

Fig. 2. DBM and cabbage looper population fluctuations in a Chinese kale seed production field at Maejo, Chiang Mai, 1989-90. Data points are means of four replications. Arrows indicate insecticide treatments for DBM and/or other species (see text).

Armyworm

Spodoptera litura is considered a major pest only during the preflowering stage. The adults appear to be abundant at the end of the rainy season when the characteristic feeding damage is often observed on foliage of cruciferous crops. Although the larvae can be destructive to young plants, they appear to cause little damage to flowers or developing seed pods; we seldom observed *Spodoptera* larvae in flower clusters or on the upper plant parts. The average number of larvae observed on the plants was highest during the preflowering stage from November to mid December (Fig. 3). Variability in the data probably reflect the sporadic occurrence of the insect's egg masses and subsequent larval clusters within the field; this irregularity might preclude inclusion of this species in any quick and simple sampling procedure.

The apparent rapid decline and disappearance of the insect after the beginning of flowering reflects more the fact that only the upper plant parts were sampled at this time than absence of the species. In addition, the decline is probably associated with a high degree of parasitism by *Snellenius* (= *Microplitis*) ? *manilae* Ashmead (Hymenoptera: Braconidae) in December. Cocoons of this parasite were first found attached to young instar *S. litura* larvae on cauliflower at Maejo in mid November and then in the experimental plots in early December. Fifteen larvae were parasitized out of 18 examined from a single cauliflower plant on 28 November 1989; six out of 10 larvae examined from 4 to 18 December in our experimental seed field were parasitized. A single individual of a small unidentified ichneumonid (hyperparasite) was also reared from a group of *Snellenius* cocoons collected from cauliflower in November 1989 at Maejo. No insecticides were applied specifically for armyworms although some hand-picking of eggs and new clusters was attempted at intervals during the preflowering period.

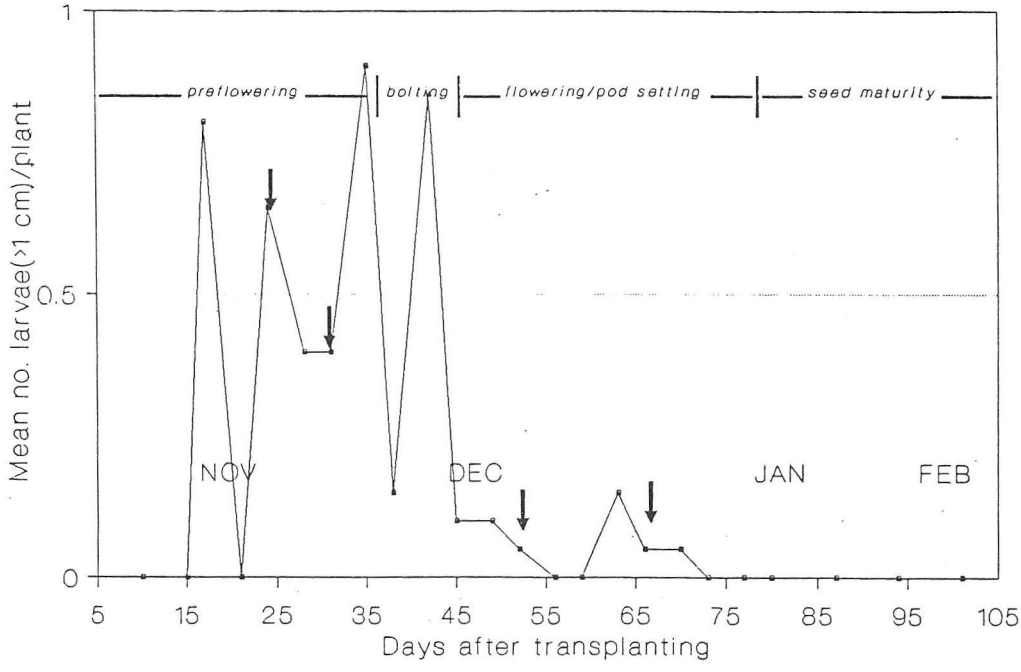

Fig. 3. *Spodoptera litura* population fluctuations in a Chinese kale seed production field at Maejo, Chiang Mai, 1989-90. Data points are means of four replications. Arrows indicate insecticide treatments applied for other insect pests (see text).

Minor Pests

Cabbage looper

Trichoplusia ni, like the armyworm, can be potentially very damaging to young plants from the seedling stage until bolting, but appears to cause little damage to the seed crop thereafter. The first *B. thuringiensis* treatment on 23 November was applied specifically for looper suppression although the count average of 0.1 larva/plant on this date was lower than the threshold which had been set at 0.3 larva/plant (Fig. 2). The crop was treated because a large number of loopers were observed outside the sampled plots. Although the threshold was exceeded at bolting in early December, these larvae were observed on lower leaves only and did not appear to be causing any serious damage to the crop. Loopers were seldom observed on the upper parts of the plants and none were counted on flowers or developing seed pods after 11 January. It is likely that natural enemies played an important role in reducing the seriousness of this pest, although no larvae were reared or examined for parasites during this study. More loopers were observed in the caged plots of the pollination experiment than in open-field plots indicating the possible exclusion of parasites and predators.

Cabbage webworm

Hellula undalis may cause occasional damage to seed fields, especially to young plants in the preflowering stage. They occurred in small numbers sporadically throughout the season. We felt it necessary to treat with insecticide once after a total of four were observed feeding on flower primordia of young plants in the sampled plots at 25 days after transplanting. Other *H. undalis* larvae were occasionally found feeding inside wilting plant stems and in late-blooming flower clusters.

Pieris brassicae

Larvae of *Pieris brassicae* L. (Lepidoptera: Pieridae) were not recorded within our seed fields during the sampling period but were frequently observed on *Brassicas* at Maejo in February and March. At this time many larvae in the field were parasitized by *Apanteles* sp.(*glomeratus* group). We reared several groups of *Apanteles* from parasitized *Pieris* larvae; *Eurytoma* sp. hyperparasites emerged from two *Apanteles* cocoon clusters. The hyperparasites emerged from 17 out of 32 *Apanteles* cocoons in one cluster and from all 15 cocoons of the second cluster.

Pontia daplidice

Two late-instar larvae of *P. daplidice* Rober (Lepidoptera: Pieridae) were found feeding on cabbage plants in a field near our seed plots on 3 January 1990. This is apparently the first record of this species from a low elevation in Thailand.

Helicoverpa armigera

Helicoverpa armigera Hübner (Lepidoptera: Noctuidae) larvae were first observed feeding on flowers just outside the sampled plots on 8 January. Although a peak of 18 larvae per 20 plants sampled was recorded on 11 January, these insects did not appear to be causing significant damage. Larvae were always found feeding on the terminal flower clusters; they appeared in the field at a time when few flowers remained and most seed pods had already formed. This pest occurred at about the same time on Chinese kale in northeast Thailand where farmers complained about the difficulty of controlling it with insecticides. Although late instar larvae are large and of obvious concern to farmers, it is not known if damage to the late-blooming terminal flower clusters results in significant yield losses. The insect's tendency to feed in the

tops of plants would appear to expose it to birds and other predators; one farmer in the northeast reported that birds often came to his seed field to feed on the large worms. He also expressed a concern that his insecticide treatments were interfering with the birds' feeding activities.

Aphids

Aphids (species undetermined) were present during the entire season from seedling stage until harvest. The threshold of 40% was exceeded four times during the sampling period. The highest level of 60% infestation (of flower clusters) occurred during early flowering on 21 December and pirimicarb was applied (together with the *B. thuringiensis* treatment for DBM on that date). Pirimicarb was again applied with a *B. thuringiensis* treatment for DBM 2 weeks later when 55% of the flower clusters were aphid-infested. Both applications were effective and aphid counts were low for 2-3 weeks afterwards. Although aphid populations were high again on plants in February during the seed maturity stage, they appeared to be causing little damage and were not treated. Larvae and adults of *Menochilus sexmaculatus* F. (Coleoptera: Coccinellidae) plus adults and 'mummies' of *Aphidius* sp. were frequently observed in the field during this final stage of the crop.

Fleabeetles

Fleabeetles (*Phyllotreta sinuata*) are potentially damaging to plants in seedbeds and to newly transplanted seedlings early in the season; later plantings are more susceptible. We recorded 0.2 adult/plant on 14 November at 2 weeks after transplanting and occasionally noted the presence of fleabeetles in the field thereafter. Protection of seedlings is recommended with granular systemics for later plantings or when fleabeetles are abundant.

Leafminers and other Diptera

Liriomyza brassicae Riley (Diptera: Agromyzidae) leafminers were present in small numbers but appeared to cause no damage to the crop. Natural enemies may control this pest when long-residual, broad-spectrum insecticides are avoided. Leafminers have occasionally damaged crucifer plantings in the North but this is thought to have occurred as the result of the destruction of natural enemies with insecticides used to control other pests.

Unidentified small dipteran larvae were occasionally found causing wilting of leaves (preflowering stage) and later wilting of terminal flower clusters. The larvae burrow and feed inside leaf petioles and young flower stalks but appear to cause little damage because of their low numbers.

Harlequin bug

Eurydema pulchrum Westwood (Hemiptera: Pentatomidae) was first observed on the last count on 8 February during the seed maturity stage. Although adults and nymphs were frequently observed on plants after this date until harvest, it is not known if they cause any damage to the maturing seed crop.

Parasites of DBM in Northern Thailand

Materials and Methods

Field collections of DBM larvae and pupae were made in March and April 1989 in preparation for rearing and release of imported *Diadegma semiclausum* Hellén (Hymenoptera: Ichneumonidae) at Maejo in Chiang Mai. The parasites did not reproduce in the high temperatures in our

laboratory, however, and none were released. Additional DBM samples were taken from crucifer fields during the 1989-90 dry season to determine what parasites were already present around Chiang Mai; four samples from each of two locations were made at irregular intervals throughout the growing season from 23 December 1989 to 22 March 1990. Samples were taken from crucifer crops at the Maejo campus (MIAT) and at or nearby the East-West Seed Company field station at Ban Chedi Mae Krua (BCMK) 10 km north of the Maejo campus. The last sample at Maejo on 22 March 1990 was taken from a destructive harvest of 200 potted cabbage plants which had received no insecticide treatments. Other crucifers at MIAT had been treated almost exclusively with *B. thuringiensis* while those at BCMK received more frequent applications of broad-spectrum materials in addition to *B. thuringiensis*.

Rearing results are summarized in Table 1. All percentages reported below are based on the parasitized fraction of the total number of parasites and adults recovered after rearing rather than the total number of DBM larvae or pupae collected, i.e. larvae/pupae which were lost to disease or other causes were not included in the denominator. For reasons we cannot explain, 20-25% of the larvae from each batch reared could not be accounted for by emerged DBM adults, parasites, or the remains of dead larvae.

In addition to samples from Chiang Mai, two collections were made from farmers' fields in northeast Thailand on 10-11 January 1990. No parasites emerged from 104 larvae and 49 pupae collected from a heavily DBM-infested mustard seed field 9 km south of Nakorn Phanom town. A single individual of *Brachymeria lasus* Walker (Hymenoptera: Chalcididae) emerged from among 107 DBM pupae collected from a heavily infested Chinese cabbage seed field 33 km east of Loei town; no parasites were found among 154 DBM larvae reared from this field. Both fields had been treated with broad-spectrum insecticides and DBM appeared to be beyond control in the field sampled at Loei.

Table 1. Parasites reared from DBM larvae and pupae from Maejo (MIAT) and Ban Chedi Mae Krua (BCMK) in Chiang Mai, Thailand 1989-90.

Date	Location	DBM collected		Parasites emerged				Adult DBM	Total recovered[a]
		larvae	pupae	*C. plutellae*	*D. collaris*	*M. orientale*	*B. excarinata*		
16 Mar.89	MIAT	–	46	–	6	0	2	38	46
16 Apr.89	BCMK	–	89	–	0	1	0	50	51
19 Apr.89	BCMK	84	–	65	–	0	–	9	74
		–	63	–	0	0	0	47	47
23 Dec.89	BCMK	200	–	20	–	0	–	149	169
			50	–	0	0	0	50	50
16 Jan. 90	BCMK	–	156	–	0	0	0	156	156
14-16 Feb. 90	MIAT	292	–	94	–	1	–	105	200
			33	–	2	0	0	20	22
21-22 Feb. 90	MIAT	178	–	58	–	2	–	68	128
			10	–	1	0	0	9	10
7 Mar. 90	MIAT	125	–	57	–	6	–	43	106
			87	–	11	10	7	49	77
16 Mar. 90	BCMK	183	–	18	–	0	–	84	102
			11	–	1	0	0	8	9
21 Mar. 90	BCMK	51	–	20	–	2	–	19	41
22 Mar. 90	KIAT	285	–	89	–	6	–	109	204
			143	–	33	5	27	42	107

[a]Total number of DBM parasites + adults recovered after rearing. This figure was used in calculating percentages of parasitism cited in the text.

Results and Discussion

Egg parasites

Although we did not sample for DBM egg parasites in the course of this study, the occurrence of *Trichogrammatoidea bactrae* Nagaraja (Hymenoptera: Trichogrammatidae) from lowland central Thailand and *Trichogramma confusum* from the Khao Khor highlands of Petchaboon Province (Keinmeesuke and Vattanatangum 1986; P. Keinmeesuke, pers. comm.) suggests that one or both of these species may also occur in the north.

Larval parasites

C. plutellae: This species appears to be the dominant larval parasite from December to March in our area (Table 1). Parasitism ranged from 12% in mid December to as high as 88% in mid April 1989 at BCMK; average parasitism for all samples was 41%. We observed relatively few parasites in the field during the critical period in December when DBM can be very damaging; economic damage to fresh market crucifers often occurs before sufficient numbers of the parasite are present. *Cotesia plutellae* populations appeared to rise together with those of the host. DBM populations declined after mid January (Fig. 2) which coincided with the period when *C. plutellae* was abundant.

Two hyperparasites emerged from *C. plutellae* cocoons during the course of this study. One individual, identified as *Brachymeria excarinata ?apantelesi* Risbec. (Hymenoptera: Chalcididae) emerged from a group of 39 *C. plutellae* pupae from parasitized DBM larvae collected on 15 February 1990 at Maejo; a second, *Brachymeria excarinata plutellae* (Joseph et al. 1972) emerged from among a group of 58 pupae from parasitized DBM larvae collected on 7 March 1990 at Maejo.

***Macromalon orientale* Kerrich (Hymenoptera: Ichneumonidae):** This species was reared from both larvae and pupae of DBM and constitutes a first record of its occurrence in Thailand (and possibly Southeast Asia). It has been listed as an important larval parasite in the Assam region of India (Chacko 1968). *Macromalon orientale*, along with *Diadromus collaris* Gravenhorst (Hymenoptera: Ichneumonidae) and *C. plutellae* were sent to Thailand from India in 1965 but laboratory rearing was not successful and no releases were made (Rao et al. 1971). Small numbers of this parasite were reared from larvae and pupae collected late in the growing season from mid February until the last samplings on 19 April 1989 and 22 March 1990 (Table 1). Parasitism ranged from 1% in February to 9% in early March with an overall mean of 2% for all sampling dates.

Pupal parasites

D. collaris: This ichneumonid was the pupal parasite most frequently reared from our samples (Table 1). Although two specimens were reared from 33 DBM pupae collected on 14 February 1990, most individuals emerged from cocoons collected rather late in the growing season in March. Parasitism of pupae sampled ranged from 9 to 10% in February to 9-31% in March with the highest percentage recorded from the 22 March sampling of potted plants at Maejo. The overall mean parasitism for all cocoons sampled was 9%. The fact that only one specimen was reared from BCMK was probably due to more frequent application of broad-spectrum insecticides at that location. Many adults of this species were observed in the field at Maejo in March of both years. *D. collaris*, like *M. orientale* and *B. excarinata* appears to occur in significant numbers only late in the dry season when temperatures are rising and DBM is relatively scarce. This species was previously recorded from the Khao Khor highlands of north-central Thailand where parasitism was 23% and 63% in February of 1985 and 1986, respectively (Keinmeesuke and Vattanatangum 1986).

***Brachymeria excarinata* Gahan:** Specimens identified as the subspecies *Brachymeria excarinata plutellae* (Joseph et al. 1972) were reared from DBM cocoons collected from late February until the last sampling of potted plants at Maejo on 22 March 1990. Parasitism of pupae collected ranged from 4 to 9% in early-mid March to 25% from the last sampling date. Overall parasitism for all cocoon samples was 6%. Although *Brachymeria* sp. parasites of DBM were imported to Thailand in 1965, they did not survive in the laboratory and none were released (Rao et al. 1971). *Brachymeria excarinata* has been recorded in southern India both as a primary parasite with 15-60% parasitism of DBM pupae (Cherian and Basheer 1939) and as a hyperparasite of *C. plutellae* (Nagarketti and Jayanth 1982).

***Isotima* sp. (Hymenoptera: Ichneumonidae):** A single female emerged on 22 March 1989 from a group of DBM pupae collected at BCMK. At least two *Isotima* species have been reared from lepidopterous rice pests in Malaysia (van Vreden and Ahmadzabidi 1986); they had not previously been recorded as parasites of DBM. This specimen readily attacked new DBM pupae in the laboratory, inserting the ovipositor through the top of the cocoon and repeatedly flexing its abdomen. This parasite probably has a wide host range and perhaps attacks DBM only when other hosts are not readily available.

Summary

Cotesia plutellae appears to be the dominant larval parasite at the two locations sampled, destroying 45-50% of DBM larvae in February and March. *Macromalon orientale* was present in low numbers in March but did not parasitize more than 9% of larvae. A total of about 60% of DBM larvae were parasitized by the two species at Maejo in early March. *Diadromus collaris* appears to be an important pupal parasite late in the growing season, parasitizing about 10% of DBM pupae in February and up to 30% in March. Although numbers of *B. excarinata* were negligible in February, this species parasitized 25% of the pupae sampled at Maejo on 22 March 1990. From 27% to almost 60% of all DBM pupae sampled were destroyed by a combination of these two species at Maejo in March 1990. The main 'gap' in the occurrence and activity of DBM parasites appears to be the critical period early in the dry season in December when farmers are most likely to treat with broad-spectrum insecticides. It is during this period when the careful choice of more selective products should greatly increase the chances of effective natural control occurring later in the season. Further development and application of basic pest management principles is urgently needed to reduce dependence on pesticides and to preserve the diversity of natural enemies associated with crucifer crops in northern Thailand.

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Integrated Pest Management of Diamondback Moth: Practical Realities

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Abstract

Diamondback moth, *Plutella xylostella* (L.), is a serious and important pest of crucifers in many parts of the world, particularly in the tropics. Although many studies have been conducted on this pest, the development of realistic integrated pest management (IPM) for it is not progressing as it should, and even less so on its practical implementation. The many reasons for this include overreliance on chemical control, overemphasis on basic and narrow-aspect research which lacks a holistic outlook, inadequate understanding of the farmer, particularly his pest perception and realistic needs, and the subtle influence of the existing socio-marketing factors. In this paper, these constraints are critically examined, with attempts also made to identify the positive steps to be taken to expedite the current initiatives in IPM development and implementation. The currently known integrating components/techniques are appraised and several common key elements important for successful IPM are identified. The latter mainly includes harnessing key natural enemy species, using microbials, and applying relatively safer insecticides when these are necessary as guided by appropriate action thresholds. Other useful but less commonly exploited elements include proper timing of planting, crop rotation, physical barriers and trapping. With respect to promoting greater IPM implementation, special emphasis is necessary to generate increased awareness and transfer of available practical IPM programs. The strategic steps will include determination of farmers' pest management knowledge, attitude and practices, IPM trial demonstrations, and appropriate training of extension personnel and farmers. Particularly in farmer training, suitable development support communication is to be utilized, encompassing pre-tested posters and pamphlets, and other audiovisual media. Also, the field school approach should be adopted.

Past Efforts in the Control of Diamondback Moth

One of the causes for the slow development of IPM program for diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera:Yponomeutidae), is the limited effort devoted to the investigations concerning this aspect. This is clearly reflected in publications (Talekar et al. 1985), wherein only 2.4% directly concerned IPM (Table 1). Of the remainder, 16.8% were of a general nature, covering descriptive, biological and ecological studies, 17.9% dealt with basic studies, and 62.9% and 65.3% on various control methods.

In general, a large proportion of the past efforts was devoted to studies of a basic nature which lacked adequate appreciation of the real field situation. Other than those which dealt directly with control aspects of DBM in the field there was little implementation consciousness or other practical considerations.

Another limiting factor is the overemphasis on chemical control. At least a third of past research efforts was concerned with insecticidal investigations, mostly (22.7%) on simple

Table 1. Relative efforts on DBM research as reflected by published articles on studies made until 1985.

Aspect of research	Published papers	
	Number	%
General		
Descriptive, biological and ecological	171	16.8
Basic studies	182	17.9
Morphology and taxonomy	15	1.5
Physiology and development	15	1.5
Biology	52	5.1
Ecology	100	9.8
Control Methods	639	62.9
Biological control	238	23.4
Predators and parasitoids	138	13.6
Microbials	100	9.8
Cultural control	24	2.4
Host-plant interaction	25	2.5
Chemical control	302	29.7
Insecticide toxicology	40	3.9
Insecticidal control	231	22.7
Insecticide resistance	31	3.1
Pheromones, juvenile hormones, chemosterilants and repellents	50	4.9
Integrated control	24	2.4

screening for effective chemicals. The remaining was on more specialized aspects and of a fundamental nature, such as insecticide toxicology and resistance studies.

Many different control components for DBM are often investigated in isolation from one another. Such a compartmentalized approach normally lacks a holistic outlook, and has hindered the development of an IPM program. Moreover, discontinuity in research activities due to frequent changes in research interest of investigators further aggravated the situation. Consequently, a sufficiently long-term aim geared towards eventual IPM development was lacking. The problem was accentuated by repetition of many short-term projects, mainly by different individuals in isolation and over different time periods. All these have in one way or another resulted in the limited development of IPM for DBM.

Status of the Available Integrating Components

Nothwithstanding that past research efforts were mostly confined to aspects not directly aimed at the conscious development of IPM, much of the knowledge generated has constituted the basis for recent IPM programs of DBM. Invariably, the separate studies on the various aspects have helped to provide deeper insights into the potential of the different elements. The depth of understanding varies, however, being largely governed by the extent and nature of investigations undertaken. Presently, numerous potential management components are known and their current status is as follows.

Biological Control

Considerable effort has gone into biological control of DBM in many parts of the world, including the use of microorganisms, predators and parasitoids, with most effort having gone into the latter.

Parasitoids: In Southeast Asia, efforts aimed at biological control of DBM by introduced parasitoids from abroad were first attempted in the 1920s by Indonesia (Sastrosiswojo and Sastrodihardjo 1986). Although the early efforts failed, subsequent attempts (Vos 1953) where *Diadegma semiclausum* Hellen was introduced from New Zealand succeeded. The parasitoid became established as an effective biological control agent around Pacet, West Java. Following this, further attempts were made to introduce it to other cabbage areas in Indonesia. Although success in establishment was achieved after several attempts, the level of parasitization during the period 1968-70 was still relatively low (<60%) and inadequate to control DBM. But firm establishment was achieved in 1971-75 when the parasitoid was commonly found in Java, and in many parts of Sumatra, though not in Sulawesi (Sudarwohadi and Eveleens 1977). The parasitism rate was well beyond 60%, and in many places averaging more than 80% and providing effective suppression of DBM.

In Malaysia two parasitoids (*Oomyzus sokolowskii* and *Cotesia plutellae*) were introduced into the Kundasang (Sabah) in the 1970s. In spite of no recoveries initially, *C. plutellae* was recently found in Sabah and is contributing significantly towards the biological control of DBM there.

Between 1975 and 1977, attempts were also made to establish three exotic species (*D. semiclausum*, *Diadromus collaris* and *O. sokolowskii*) into the Cameron Highlands (Ooi and Lim 1989) because the existing parasitoids (*C. plutellae* and *Tetrastichus ayyari*) could not provide full DBM control (Ooi 1979; Lim 1982). However, only the first two have become established and have dispersed all over the Cameron Highlands. Presently, the parasitoid complex, consisting mainly of *D. semiclausum*, *C. plutellae* and *D. collaris*, serves as the core element and foundation of an IPM program for DBM, which is being gradually transferred to highland farmers (Lim et al. 1988).

Biological control of DBM is also given importance in Thailand. Emphasis is placed mainly on augmentation, particularly on mass releases of egg parasitoids (*Trichogramma* spp.). By releasing *Trichogramma confusum* at the rate of 375,000 parasitoids/ha in the highlands of Pechaboon Province, the field parasitism can be greatly increased, reaching up to 65.5% during 1987-88 (Vattanatangum 1988). Another species of potential utilization is *Trichogrammatoidea bactrae*. Presently, studies are being intensified on how to use these agents more effectively, particularly in the lowlands.

Outside Southeast Asia the importance of parasitoids in controlling DBM has also been clearly demonstrated. In Taiwan, for instance, in addition to *C. plutellae* the newly introduced *D. semiclausum* has become established in the crucifer-growing areas in the highlands (Talekar 1990). In these areas all farmers have reported considerably less DBM damage. Consequently, there was also very little use of insecticides except *Bacillus thuringiensis*. Based on insecticidal expenses for DBM control before and after parasitoid introductions, this biological control project represents potential savings of over US$365,000 per year. In addition, environmental contamination is also reduced because of less insecticidal inputs.

The introductions of parasitoids for the effective suppression of DBM have also been achieved in New Zealand (Muggeridge 1939) and Australia (Goodwin 1979; Waterhouse and Norris 1987).

In Zambia it was claimed (Yaseen 1978) that a combination of *C. plutellae*, *D. collaris* and *O. sokolowskii* have provided an 80% reduction in damage by DBM. On Cape Verde Islands, DBM, once the most important cabbage pest until 1981, is now scarce following the introdution and establishment of *C. plutellae* and *O. sokolowskii* (Cock 1983). The encouraging results were, however, also assisted by the use, where necessary, of *B. thuringiensis*.

The effective contributions of parasitoids are particularly evident in areas where the infestations of DBM are generally low, as in many parts of Canada, Europe and the United States (Muggeridge 1939; Hardy 1938; Sutherland 1966; Oatman and Platner 1969). In England, only very occasionally were large economic losses involved, resulting from mass immigration (French 1965). In Germany, France and Italy, DBM is not present in sufficient numbers to be a serious pest, and in the last-named country it is comparatively rare (Muggeridge 1939). DBM appears to be held in check in most of these regions by effective parasitoids, and Marsh (1917), in outlining the situation in the United States, pointed out that DBM was a striking example of a potentially serious pest normally held in repression naturally by parasitoids.

There is now overwhelming evidence that parasitoids do play a dominant role in the population dynamics of DBM (Lim 1986; Waterhouse and Norris 1987) and these must be given prime consideration in any management program of the moth. While full population suppression through these biological control agents is the prime objective, a partial biological control to be used in conjunction with IPM programs with attendant reduction of chemical usage is also considered important (Ooi and Lim 1989).

Predators: Among the natural enemies of vegetable pests, predators appear to be least studied and understood. In most cases, they merely constitute a listing of species. For example, spiders, coccinellid beetles, pentatomid bugs, phytoseiulus mites, chrysopids and *Ophionea* beetle were reported to attack DBM in Vietnam. These tend to build up only in the later part of the crop and can cause up to 70% prey mortality (Vu 1988). In Malaysia, syrphids, wasps and spiders are common predators (Ooi et al 1990). Ooi (1979) observed that syrphids will readily predate on DBM in cabbage fields, as was also noted by Robertson (1939) and Ullyett (1947), while Yasumatsu and Tan (1981) reported that the vespid wasp *Ropalidia sumatrae* frequently attacks DBM larvae in lowland crucifers.

In general, predators have been suggested to be important stabilizing agents for DBM populations (Ullyett 1947; Yamada and Yamaguchi 1985). Nemoto et al. (1985) explained that the higher numbers of DBM in their insecticide treated fields was because of lower predator numbers, while Sivapragasam and Saito (1988) suggested that large unknown mortalities in life table studies may be attributed to predators. Using a biological control check method, Keinmeesuke et al. (1988) demonstrated that 68% or more of DBM larval mortality was due to various predators including birds. Although predators have been suggested as major mortality factors, they presently are receiving little attention. More in depth studies are thus desirable.

Microbials: The use of microbial agents for controlling DBM has progressed most with *B. thuringiensis* where it is highly effective against the larvae. Many commercial formulations are now available and used. Recently, more promising activity is also obtained with the liquid formulation. However, in Vietnam the common presence of bacteriophage is noted to pose some constraints to using this microbial agent.

Because of increasing difficulties in achieving effective control with most chemical insecticides due to resistance development, there is in recent years more intensive and widespread use of *B. thuringiensis*. This has enabled better survival of parasitoids. Consequently, a greater abundance of both *D. semiclausum* and *D. collaris* has been observed in the Cameron Highlands in Malaysia, as well as trichogrammid egg parasitoids in Thailand.

Experimental studies with viruses have shown them to be potentially useful in the control of DBM. The main ones include granular virus (GV) and nuclear polyhedrosis virus (NPV). Among the latter are *Autographa californica* (AcNPV) (Vail et al. 1972) and *Galleria mellonella* (GmNPV) (Abdul Kadir and Payne 1989). Studies have shown that the GV is most pathogenic to DBM while GmNPV can kill faster.

Currently the practical employment of viruses for the control of DBM still faces a number of problems and needs further investigation. These encompass virus production, spray application techniques and formulation.

In the case of other microbials, the main ones are *Zoophthora radicans* and *Beauveria bassiana*. These two agents are considered to be particularly useful in Vietnam (Vu 1988). In Malaysia, *Z. radicans* is also considered an important mortality factor of DBM (Ooi 1981). Generally, as the host population increases, the infection level also rises. Under the tropical conditions of Asia where there is abundant rainfall and a high mean temperature these agents normally serve as a constant mortality factor.

Use of Plant Resistance

Plant resistance is a highly useful strategy that can be applied in the control of pests. It does not require any special action from growers and constitutes a cheap and practical input in the integrated control system.

In the case of DBM some specific components for resistance/susceptibility have been identified: allyl isothiocyanate, glucocheirolin, glucoerucin, gluconapin, gluconasturtiin, gluconringiin, glucotropaeolin, progoitrin, sinalbin and sinigrin (Hillyer and Thorsteinson 1969). Although differences in varietal resistance of crucifers to the moth are now known (Rudder and Brett 1967; AVRDC 1976; Dickson et al. 1986) the more tolerant varieties are generally not preferred because of other poorer agronomic features. Consequently, these cultivars are not generally cultivated.

Cultural Practices

Time of Planting: Because pest abundance can be greatly influenced by seasonal factors (e.g. rainfall) the time of planting may sometimes govern the final performance of a crop. For instance, DBM infestations are observed to be generally lower during the wetter period (Lim 1982). Thus, avoiding the cultivation of crucifers in the drier parts of the year has ensured less need for insecticidal inputs to control DBM.

On the other hand, disease occurrence on vegetables is generally higher during wet periods. Thus, for specific location and crop it is important to determine appropriate sowing time to mitigate the envisaged problem so as to obtain the maximum production.

Crop Rotation: This is one of the most effective cultural measures for reducing monophagous or oligophagous insects such as DBM as in Thailand (Vattanatangum 1988). It is also widely practiced in Vietnam, especially in larger commercial cultivation. In upland areas the rotation is mainly among crucifers, cucurbits and beans. Two common rotational systems are: cabbage-peas-turnip or cabbage-luffa, and tomato-turnip or cabbage-squash or cucumber (Vu 1988). By this means, DBM on crucifers can be suppressed substantially.

Intercropping/Mixed Cropping: Rational intercropping of various vegetables maximizes the use of rotation and this can help localize the spread of many arthropod pests.

In both Philippines and Malaysia, tomato intercropping with cabbage was observed to reduce DBM larval infestations (Buranday and Raros 1975; Sivapragasam et al. 1982), possibly due to volatile compounds which have a repellent effect on adult DBM. In some instances this has resulted in higher cabbage yield (Embuido and Hermana 1981). However, inconsistent results of others (Magallona 1977) suggest the need for further investigations.

Alternative Food Source and Shelter Plants

Many adult beneficial insects often require foods such as honey and/or pollen. Refuge or shelter plants may also be important for their survival, particularly in avoiding excessive pesticide sprays. For the key parasitoids of DBM, some plants found to be important have included many species of wild flowering plants and cultivated legumes (e.g. beans and peas) (Lim 1982). The more important wild plants are *Malastoma malabathricum*, *Crotalaria* spp. and *Cleome*

rutidosperma. These plants are found capable of increasing greatly the lifespan of the parasitoids. In practice, encouraging the planting of such useful plants in the crop vicinity would be desirable.

Sanitation: Sanitation is a simple, but important preventive measure of pest control. Even simple removal can greatly reduce the infesting potential. Removal may also include alternate hosts, weed hosts, volunteer plants and crop residues. For instance, some farmers in Thailand practice destroying crop residues to disrupt the development of DBM (Vattanatangum 1988).

Fallowing and land drying can also be critical. In Vietnam, for example, the land is usually plowed over and left exposed to the hot sun for at least a week prior to cultivation. This helps in cleaning up sources of DBM as well as improving general soil conditions (Vu 1988).

Regulated Irrigation: Regulated irrigation with sprinklers has been demonstrated to be capable of reducing substantially the infestations of DBM (Talekar et al. 1986). Here, the water sprays interfere with mating and oviposition of the moth. In addition young larvae may also be drowned during periods of heavy rain.

Physical/Mechanical Methods

Some physical and mechanical methods have also been explored in Thailand in relation to DBM control (Vattanatangum 1988). The blue-light traps, for instance, are capable of capturing large numbers of adult DBM. Planting crucifers such as Chinese kale, pakchoi, Chinese cabbage and cabbage under fine-mesh netting houses also gave satisfactory yields. There was good protection from DBM as well as many other common pests. This control method is now being further investigated in the central plain of Thailand where DBM is rapidly developing resistance to nearly all insecticides.

The use of yellow sticky traps in conjunction with other conventional methods has also been reported to be effective in controlling DBM in Thailand.

Recent and Novel Techniques

These techniques include the use of pheromones, chitin inhibitors or insect growth regulators (IGRs), chemosterilants, antifeedants, and sterile male release, and botanicals.

Except for IGRs, pheromones and some botanicals which are being given increasing attention recently, most of these techniques are still exploratory and are presently of no practical use. A few IGRs have so far been used against DBM but are already facing problems similar to those of conventional insecticides.

Natural products are also receiving increasing attention for possible use against DBM. For example, in Thailand, extracts of neem (*Azadrachta indica*) have been found effective and used against DBM on Chinese kales and cabbage. In Malaysia and the Philippines exploratory studies have also been initiated.

Chemical Pesticides

Currently, chemical insecticides still constitute the main control tactics for DBM in most parts of the world where this pest is serious. A wide range is available and are being used, often indiscriminately and resulting in many undesirable problems.

In terms of using chemical insecticides there is an urgent need to refocus their employment towards a supplementary function, and integrating them within a more holistic IPM approach. To enable them to be used more prudently, the investigatory aspects will need to include identifying more selective chemicals, improvment in application technology, and applying wide-spectrum chemicals to achieve ecological selectivity, such as correct time and method of application, use of minimal effective dose, and applying appropriate formulations.

Attempts in IPM Programs

It is now clear that many diverse and potential elements for IPM development against DBM are now available. Unless some of these are assembled together into appropriate IPM programs the integrated approach to DBM management will not fully materialize. In this regard, it is encouraging to note that some efforts have already been initiated, even though still limited presently (Table 2).

In all these efforts many common features are evident, the more important ones being:

(1) Only a few of the potential elements have so far been practically incorporated.
(2) The most common components used are biological control (particularly parasitoids), action thresholds and monitoring, and judicious use of chemical insecticides when the pest thresholds are exceeded. In a few cases there have been additional elements incorporated such as crop rotation, proper timing of planting, light trapping and use of physical barrier.
(3) In general, none of the IPM programs are inferior to the existing prophylactic control methods presently practiced by farmers. Although crop yields may not always improve substantially, they generally are not less.
(4) In cases where the thresholds are exceeded and insecticidal applications necessary, both the frequency and amounts of insecticides used are substantially reduced.
(5) For most of the IPM program, *B. thuringiensis* has been incorporated, serving as a replacement to many broad-spectrum insecticides and providing the needed selectivity when quick action by chemical intervention is necessary.
(6) In terms of profits there is usually a substantial increase, mainly because of savings from the enormous reductions in chemical inputs.
(7) Most of the IPM programs are presently still at the experimental stage. Although there has been field adoption, this occurs only on a limited scale.
(8) The IPM programs developed so far are still largely executed and confined within the domain of researchers. There is presently inadequate involvement of both the extension personnel and the farmers.

The overall attempts devoted to the development and implementation of IPM for DBM are in general still limited. Nonetheless, there have been many useful lessons gained and these can serve as important guides towards future IPM activities for DBM.

Discussion

The limited attempts of IPM for DBM have demonstrated that not only are such IPM programs feasible but there are also many benefits. In spite of this, IPM is not widely adopted. This is largely due to several constraints which must first be overcome. To expedite the current initiatives in IPM development for DBM and its implementation, a number of positive steps will need to be taken.

Firstly, since self-regulating processes to maintain stability must be present for an IPM program to have maximum and long-lasting impact, and several key DBM parasitoids can well satisfy this role, introductions of these species into areas where they are absent should be an important first step. The current effort of AVRDC (Talekar 1990) in establishing these parasitoids in Southeast Asia is therefore to be encouraged.

Experiences to date have shown that biological control agents are the core components in IPM programs of DBM. These therefore must not be disregarded. Presently, the main biological control agents are mostly parasitoids while the microbial is mainly *B. thuringiensis*. The role of predators and how they may be specifically employed is however, still unclear. Until further studies are made on how to use them, all efforts should be made to encourage conserving them as much as possible.

Table 2. Some attempts at IPM[a] of DBM and their performance.

Country	IPM elements which are incorporated	Status	Benefits	
			Pesticide reduction	Profit
Indonesia[b]	Key parasitoids, *B. thuringiensis*, selective chemicals, simple monitoring, and use of action threshold.	Farm level	Types of insecticides, dosages and frequency greatly reduced.	Not available
Malaysia[c]	Avoid planting in drier period, key parasitoids, simple monitoring, use of action threshold, *B. thuringiensis*, selective chemicals.	Experimental, some at farm level. Now in process of transfer to farmers for adoption.	Number of applications reduced from 7 to 3. Amount chemical used reduced by 98% and expenses by 97%.	Crop yield increased by 22% and profit by 84%.
Taiwan[d]	Establishment of the key parasitoid *D. semiclausum* to supplement other important ones such as *C. plutellae* and *D. collaris*. Where sprays are needed, *B. thuringiensis* was encouraged.	Cabbage fields of farmers	Use of insecticides greatly reduced. Where used, they are mostly *B. thuringiensis*.	Estimated potential savings of over US$370,000 per per year.
Thailand[e]	Key parasitoids, monitoring, use of action threshold, selective chemicals, in some cases also under fine-netting and use of light traps.	Experimental, some at farm level, Now in process of transfer to farmers for adoption.	Amount of pesticides greatly reduced. Cost of insecticides spent reduced by 80%.	Profit tripled.
Vietnam[f]	Crop rotation (crucifers and cucurbits), separate nursery, main planting intersown with tomato, *B. thuringiensis* and other microbials, selective chemcials, monitoring and action threshold.	Now in process of transferring to farmers.	Pesticide inputs reduced by 51%.	Profit figures not available. However, pest infestations are greatly reduced, by 8-10 times when compared to farmer practices.

(Continued)

Table 2. Concluded

Country	IPM elements which are incorporated	Status	Benefits	
			Pesticide reduction	Profit
USA				
Lower Rio Grande Valley[g]	Insect sampling and action threshold (of (0.3 larva/plant) to manage complex of lepidopterous cabbage pests including DBM.	Large-scale tests in commercial cabbage fields.	On average two fewer insecticide applications were required.	Greater market-yields
Hawaii[h]	Parasitoid *C. plutellae* and overhead sprinker irrigation system for DBM, and predators and timely applications of insecticides for other associated important pests.	Commercial farm level.	Insecticide inputs reduced substantially.	Commercially cost effective. Chemical control costs reduced by 89%, while production increased by 93%.

[a]Establishment of key parasitoids leading to effective suppression of DBM is also included since parasitoids are regarded as the cornerstone of DBM IPM. [b]Sastrosiswojo and Sastrodihardjo 1986; [c]Lim et al. 1988; [d]Talekar 1990; [e]Vattanatangum 1988; [f]Vu 1988; [g]Cartwright et al. 1987; [h]Nakahara et al. 1986.

Up to the present time, most DBM management practitioners have never needed or been encouraged to think in terms other than chemical. This must change. They should be made more aware of the need for IPM. Until the awareness attained is adequate, IPM can only expand slowly. In particular, special emphasis is necessary to generate increased awareness and transfer of available practical IPM programs. The strategic steps will include IPM trial demonstrations and appropriate training of extension personnel and the farmers. Particularly in farmer training the field school approach should be adopted, wherein hands-on training is to be conducted under actual farm situations.

Training must include practical field skills as well as delivering only relevant knowledge. Communication should use an appropriate variety of media channels in strategic campaigns to reach well-defined target audiences with specific messages whose impact can readily be measured. In particular, suitable development support communications are to be utilized, encompassing pre-tested posters and pamphlets, and other audiovisual media.

Pertaining to training and generating increased IPM awareness, regular follow-up visits on progress are particularly essential. This, however, can only be effectively achieved if there is adequate support of extension services. The latter is presently still very weak in many parts of the developing tropics where DBM is also most serious. An urgent need, therefore, is for increased government support to ensure an efficient extension program on IPM implementation for DBM.

Since vegetables are generally marketed through the private sector rather than government channels, promotion through social marketing campaigns aimed at all parts of this system should constitute a special necessary element of IPM for DBM.

In terms of research on DBM this must be pursued holistically. For too long there has been overfocusing within the confines of the different IPM components, wherein the research is most of the time conducted in isolation. This should now be avoided. Of special importance is that

there must be a true integration of research in developing IPM programs. Specifically, assembling and further improving appropriate IPM programs of DBM must be given prime focus.

To ensure practical impact research must also involve close collaboration with farmers. There are certainly crucial needs for both pure and applied science aimed at improving human life through improved crop protection. But practical requirements must be paramount in much of the developing tropics, especially where there are crisis conditions of food production (Way 1982) and excessive use of chemical toxicants as in the present case of DBM. Certainly while specialist IPM research needs to be continued and strongly supported, there must be adequate effort directed to involve vegetable growers in order to establish those elements of IPM that are likely to be useful in practice (Way 1985).

To date, most research programs on DBM management have been centered only on the biological aspects. But IPM technology adoption extends well beyond this. Thus, efforts must now also be directed at such areas. Although many social and marketing factors can influence pest control decisions for DBM, presently still very little is known of these aspects. In particular, it is critical that studies be initiated to understand the farmers better, such as their knowledge, perception, attitude, and practice relating to DBM. Arising from these will then be a truer understanding of the farmers' aspirations and constraining factors. This will better ensure that any follow-up research to be undertaken will consider the practical realities of the farmers. It will also improve the communicative processes crucial for translating IPM for adoption.

Now that feasible IPM programs for DBM are available, increased efforts must be devoted to speed up implementation of these programs. The primary goal should concentrate on the elements of transfer, that is, simplifying, assembling, delivering, monitoring, and evaluating the IPM of DBM. In this regard there must therefore also be adequate integration of people besides that of biological elements. Unless the key groups of people involved (in particular researchers, extension workers, farmers and the socio-marketing sectors) are well integrated, implementation of IPM for DBM cannot be expected to progress satisfactorily and rapidly. There should also be emphasis to ensure sustainable institutional support for the IPM programs. Only through such sustained efforts can IPM of DBM truly emerge to become a practical reality to a large number of vegetable growers, and bring a previously thought-to-be inaccessible technology to the many illiterate resource-poor farmers so that they may receive the many benefits of IPM. Of particular importance is the opportunity to free themselves from pesticide dependency, and reducing insecticidal inputs along with the many undesirable problems commonly associated with the overuse of chemical insecticides in DBM control.

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It is a challenge to summarize a meeting as diverse as this one. Thankfully it has been held together by a common thread. A silken thread, perhaps, spun by a larva of *Plutella xylostella*, defiantly clinging to a glossy leaf, covered in chemical residue and fungal spores, out of sight of a foraging parasitoid, for a moment at least. That common thread which ties us all together here has been, perhaps, a lifeline at times for each of us as we try to gain and hold onto an understanding of such heavy subjects as ichneumonid taxonomy and biochemistry of resistance mechanisms. It would have been much easier to hold concurrent sessions, so that we could each concentrate on the familiar and avoid the unknown. But this would have denied us one of this workshop's greatest achievements. No one here now has an excuse to ignore each other's approach to DBM management nor, after the last session's papers in particular, to seek single technology solutions to our common problem.

In the five years since the last workshop, DBM and its lepidopteran relatives like *Trichoplusia*, *Hellula*, *Crocidolomia*, *Pieris*, and others, have been at least as active as we have. The problem they pose has grown, it has spread, and the miracle cures of the past, such as IGRs and *Bacillus thuringiensis* have shadows hanging over them today. To find a silver lining in that cloud, I think it is significant that the appearance of DBM resistance in North America has brought the full force of American entomological expertise to bear on the problem, as we have seen so well represented at this meeting. I am sure their contribution will be of great benefit to ongoing programs in the tropical world.

We began this workshop learning about advances in the components of integrated pest management for *Brassica* pests, the elements of the strategies which we put together today. We also learned, in a few short papers, how little we really knew about the behavior and ecology of DBM, its movement (dare I say migration) and its mating behavior, and how much less we knew about the other moth pests on cruciferous crops.

The potential for plant resistance was made clear, specifically through the protection conferred by leaf glossiness, with all its complex genetic sources. The potential to put the strong antibiosis demonstrated into other *Brassica* lines must certainly exist.

Another technology, pheromone trapping, has pervaded this workshop. How many slides have we seen of a *Brassica* crop with a little pheromone trap emerging above the canopy? The use of pheromone traps to aid research and to gain a basic understanding of pest phenology seems now to be a standard tool. We should not underestimate how much of an advance this is. But beyond this we have heard how, in Japan, the use of pheromones for mating disruption has moved from elegant theory to practical reality. All we need now is to get the price down.

Our discussions of microbial pesticides control focused, most appropriately, on *B. thuringiensis*. I would not have thought, 10 years ago, that *B. thuringiensis* would be so popular today. As a purveyor of parasitoids and predators, I would have dreamed it, but not seriously thought it would come true. We seem now well into our second generation of *B. thuringiensis* technology. At this workshop we have heard of extensive and impressive trials of genetically engineered *B. thuringiensis*, and of the particularly exciting development of putting *B. thuringiensis* genes into long-lasting bacterial cells for foliar application. We skirted the issue of engineering *B. thuringiensis* into crucifers, which I take as a collective message of silent concern.

The clouds gathering over the use of *B. thuringiensis*, with the demonstration of resistance in Hawaii and elsewhere, are most worrying. We all want to know how local and stable this resistance might be and whether new genetic technologies can really keep us one step ahead of DBM's impressive physiology. But we have been reminded as well that there are other promising pathogens, fungi, and viruses, which deserve more study.

I am conscious of a personal bias towards the kinds of biocontrol agents which you can see and count. However, even in a field I thought I knew well, I have learned a lot. We have seen the use of parasitoids chronicled in the Pacific, in Asia, in the Caribbean and on a very small island off Africa. We then learned that all of the parasitoids which we have spread around the world may not be what we thought they were after all. As always, we come back to the taxonomist, grudgingly and late, for help.

Most heartening has been the very real and substantial success of parasitoids like *Diadegma semiclausum* in the highlands of Taiwan and Malaysia, and the real efforts to spread other useful parasitoids into farming systems by demonstration trials and by encouraging farmers to use microbials to assist their establishment. The hot lowlands, however, remain a challenge, and point to a need for further study of new agents from warmer regions. The diversity of parasitoids which we have seen described from Romania, at the heart of DBM's region of origin, is most encouraging. Finally, we are left with an awareness that in parasitoids and pathogens we have only a part of our biocontrol armory, and that predators now deserve our attention too.

All of these challenges will be better met by the establishment during this workshop of a Global Working Group on DBM within the International Organization for Biological Control (IOBC).

Throughout the workshop, there have been frequent reminders of the potential antagonism between pesticides and natural enemies, ranging from detailed studies of pesticide effects to the salient lesson of the Cameron Highlands, where pesticide use delayed for so many years the benefits of *Diadegma* which that region now enjoys.

As our pesticide armory dwindles, it is refreshing to learn of the continuing promise of neem extracts, and progress with that and other selective pesticides. But it is significant that the part of this workshop dedicated to chemical control was so dominated by the subject of resistance. In a series of detailed papers, we have seen research groups around the world try to piece together the patterns of resistance. The intricacies of cross resistance within the insect growth regulators, the organophosphorus and other groups point to a great range of mechanisms and some continuing disagreement. But one thing is clear, there are many processes and much variation governing the stability of resistance. This, and the apparent extreme localization of resistance, for instance of *B. thuringiensis* resistance in Hawaii and chemical pesticide resistance in Florida, gives hope to resistance management.

Many presentations took this positive approach, providing us with strategies, and even evidence of resistance management. It was also significant that the International Organization for Pesticide Resistance Management (IOPRM) chose this workshop to hold its first meeting on the development of programs for pesticide resistance management on DBM. We are anticipating considerable progress in the programs to be established by this new organization.

We reached the final session on IPM with many IPM tools and technologies, including perhaps some which we thought we might have lost. These methods are being cleverly woven into IPM programs around the world. Many exciting local innovations have been introduced in these IPM packages, alongside the methods we learned about earlier in this workshop. The striking success of mustard intercropping in India, and the potential for yellow sticky traps are examples.

We have heard of activities in several countries towards development of thresholds for pesticide application against DBM, with emphasis on 'softer' pesticides, where necessary, and the incorporation of natural enemy action into spray decisions. One has a feeling of it all beginning to come together. So that we do not grow too complacent, however, we have been reminded as well that IPM is a strategy which, in the end, must be implemented and not just studied. We have seen some, perhaps too little, involvement of farmers in the development of appropriate IPM methods. The economic bottom line of IPM has been driven home in farming communities as diverse as southwestern USA and Malaysia, and we are left with the clear challenge for some years hence, of coming back to our next workshop with improved, more practical IPM programs out of the hands of researchers and into the hands of farmers.

I would like to close by saying how impressive were the contributions and level of discussion, the high quality of research reported with the willingness to share information. We owe thanks to our hosts in Taiwan, and to the organizers of this workshop.

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SUBJECT INDEX

(unless species name stated otherwise, all index words apply to diamondback moth, *Plutella xylostella*)

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