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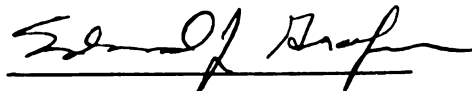
Impact of Pesticides on the Diamondback Moth,
Plutella xylostella (L.) and Effects on its
Biological Control Agent, Diadegma insulare (Cresson)

presented by

IDRIS BIN ABD.GHANI

has been accepted towards fulfillment
of the requirements for

M.S. degree in Entomology


Major professor

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**IMPACT OF PESTICIDES ON THE DIAMONDBACK MOTH,
PLUTELLA XYLOSTELLA (L.) AND EFFECTS ON ITS
BIOLOGICAL CONTROL AGENT, DIADEGMA INSULARE (CRESSON)**

by

IDRIS BIN ABD. GHANI

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**Submitted to
Michigan State University
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ABSTRACT

IMPACT OF PESTICIDES ON THE DIAMONDBACK MOTH, PLUTELLA XYLOSTELLA (L.) AND EFFECTS ON ITS BIOLOGICAL CONTROL AGENT, DIADEGMA INSULARE (CRESSON)

by

Idris Bin Abd.Ghani

Diamondback moth (DBM) is the most serious pest of crucifers. Control failure is attributed to its high capability of developing resistance toward pesticides. The impact of pesticides commonly used in cabbage fields for controlling DBM and the effects on biological control agent, Diadegma insulare, was assessed. In the laboratory, none of the insecticides tested caused 100% mortality to DBM larvae, but most were more toxic to D. insulare than to DBM adults (except Bacillus thuringiensis (Bt) Berliner var. kurstaki and esfenvalerate to DBM larvae and adults, respectively). The immature stages of D. insulare were indirectly killed (except Bt to the pupae) and were more sensitive to insecticides than DBM. Bt was toxic to both parasitized and non-parasitized larvae. The fungicide chlorothalonil was safe to D. insulare. In the field, all insecticides tested were equally effective against DBM. DBM parasitism by D. insulare was severely reduced by insecticides in the field cages but not in the plot experiments. Approaches that favor D. insulare and ensure the success of integrated DBM management programs are also discussed.

To my wife.....

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INTRODUCTION

The diamondback moth (DBM), Plutella xylostella (L.) (Lepidoptera: Yponomeutidae), is an oligophagous insect and feeds on plants that contain mustard glucosides (Thorsteinson 1953). Important economic crops with mustard glucosides are members of the family cruciferae and are grown both in temperate and tropical regions. DBM was first recorded as a pest in 1746 (Harcourt 1962). Since then there have been many accounts of its importance. In some countries such as Argentina, Australia, New Zealand and South Africa, DBM caused serious economic losses to cruciferous crops well before 1930 (Muggeridae 1930). Robertson (1936) noted that although little detailed work appears to have been done at that time, the moth was recorded as a pest of crucifers in many part of the world.

DBM has been reported from Indonesia (Ankersmit 1953, Vos 1953), Bulgaria (Khristova 1957), Malaysia (Henderson 1957), Egypt (Hassanein 1958), the Philippines (Capco 1959), Canada (Harcourt 1962), Argentina (CIE 1967), India (Abraham & Padamanabha 1968) and Taiwan (Hsu & Wang 1971). Altogether up to 128 countries report the occurrence of this pest (Salinas 1972). According to Cartwright et al. (1987), DBM is a cosmopolitan pest of Brassica crops and a key pest of cabbage in south Texas.

In general, the level of DBM infestation varies considerably according to the locality, conducive environment, length of growing season, number of acres of cruciferous crops grown and frequency of insecticide application (Lim

1986, Yamada & Koshihara 1978, Sun et al. 1978). The insect apparently does not overwinter in Canada (Harcourt 1986, Smith & Sears 1983) and in Japan (Yoshio 1987). There is no report on DBM overwintering in the northern United States. However, Harcourt (1986) suggested that annual infestations arise from adults which disperse from winter breeding sites in the southern United States and are carried northward in the spring by favorable winds and are favored by high temperature and low rainfall. Generally, these migrants arrive in southern Ontario during the last half of May. Chu (1986) estimated that DBM can fly continuously for several days as far as 3,000 km. Mass migration of DBM also is reported from Finland to England and other parts of Europe with distances over 3,680 km (French 1967, Lokki et al. 1978).

Control of DBM by conventional chemical pesticides has so far been the most popular method practiced by the majority of farmers throughout the world. Insecticides, including pyrethroids (cypermethrin, deltamethrin, permethrin and esfenvalerate), insect growth regulators (chlorofluazuron), carbamates (carbaryl and methomyl), and organophosphates (metamidophos, dictyophos, triazophos and methyl parathion) are commonly used (Ho 1983, Liu et al. 1981, Magaro & Edelson 1990, Cheng 1990, Leibee 1990, Shelton et al. 1990). This unilateral approach and overreliance on chemicals has resulted in the development of insecticide resistance by DBM (Ooi 1986). Resistance of DBM to DDT and BHC was reported during the late 1950s (Henderson 1957). Organophosphates and carbamates were used as alternative insecticides and again resistance problems arose within a few years (Ho 1965). Pyrethroids were used extensively to replace or alternate with the above-mentioned insecticides. DBM has also been reported to be resistant to these pyrethroids (Sun et al. 1981, Georgiou 1981, Miyata et al. 1982, Chen & Sun 1986, Tabashnik et al. 1987). Liu et al. (1981) found that DBM resistance to diazinon

(organophosphate) showed significant cross-resistance to the pyrethroids such as permethrin, cypermethrin, deltamethrin and esfenvalerate. The occurrence of multiple resistance to many kinds of insecticides has also been reported by Liu et al. (1982) and Cheng (1988). They found that this phenomena was probably due to the presence of nonmetabolic mechanisms of resistance in addition to the microsomal functional oxidase (mfo) enzymes. DBM also has been reported to be capable of developing resistance faster to most toxic insecticides like synthetic pyrethroids than less toxic insecticides like carbamate (Cheng 1988). This resistance resulted primarily from reduction in cuticular penetration, increase in detoxification and insensitivity of the site of action. Resistance to chitin inhibiting insecticides (insect growth regulators) such as chlorfluazuron and teflubenzuron has also been reported (Perng et al. 1988, Fahmy & Miyata 1990, Fauziah et al. 1990). Fahmy & Miyata et al. (1990) reported that DBM resistance to insect growth regulators gives broad spectrum cross-resistance to various types of insecticides.

Different approaches have been tried to overcome the resistance developed by the DBM to almost all insecticides used. For instance, microsomal oxidase inhibitors such as piperonyl butoxide and DDT-dehydrochlorinase inhibitors have been added as synergists but results were unsatisfactory (Liu et al. 1982a, b). Another alternative was the microbial insecticide Bacillus thuringiensis Berliner var. kurstaki (Bt) (Varma & Gill 1978, Sastrosiswojo & Sastrodiharjo 1986, Sastrodiharjo 1986, Lim et al. 1986, Kirsch & Schmutteler 1988, Tabashnik et al. 1990). However, resistance to Bt has also been reported (Tabashnik 1991, Tanaka 1991). Tabashnik et al. (1991) also found that resistance to Bt by DBM is more persistent than to chemical pesticides like esfenvalerate. The only long term advantage of Bt over chemical insecticides is that it has fewer adverse effects on the predators and

parasitoids of DBM. This is because Bt must be ingested to be effective (Fast and Donaghue 1971) and specific gut conditions are required for toxicity. Bt resistant DBM larvae may lack the high pH in their intestine necessary to dissolve the spore crystal or the intestinal proteolytic enzymes of resistant DBM may destroy the toxin or fail to "activate" a protoxin (Rogoff & Yousten 1969).

Because it is safe to the environment and to beneficial insects, there is much research being carried out to find ways to increase Bt effectiveness. Soares (1990) reported that a new Bt (MVP) in which the delta-endotoxin is biocapsulated within the bacterial cell performs 5-6 times better than the older Bt formulations such as Dipel or Javelin.

In spite of the wide adaptability of DBM towards different environments and insecticides, it appears to be held in check in some regions. 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 by parasitoids. The occurrence of such natural control of DBM is also recorded in many other places: Canada (Harcourt 1960), New Zealand (Robertson 1939), Russia (Kopvillem 1960) and Malaysia (Ho 1965). Previous records suggest that DBM may become a serious pest only where the natural enemies are absent or ineffective (Lim et al. 1986). The length of growing season, large number of acres of cabbage planted and frequent application of insecticides significantly reduce the effectiveness of DBMs' natural enemies (Yamada & Koshihara 1978, Sun et al. 1978).

For the foreseeable future, insecticides will continue to remain a powerful and essential tool in DBM management. However, their use must be minimized to prevent the present trend from reaching a 'disaster phase'

(Smith 1969). A prime strategy is to build a broader ecological base that would make possible integration of various pest management techniques.

Biological control is a natural ecological phenomenon and can potentially provide a relative harmonious, economical and permanent solution. The study of DBM parasitoids and predators has been a priority (Ooi 1986, Miko 1986). The role of larval parasitoids such as Apanteles plutellae Kurdjimov, Microplitis plutellae (Muesbeck) (Hymenoptera Braconidae), Diadegma eucerophaga (Horstmann) (Hymenoptera Ichneumonidae), D. insularis Muesbeck, D. insulare (Cresson) and Thyraeeca collaris (Gravenhorst) (Hymenoptera: Ichneumonidae) has been reported by Goodwin (1979), Chelliah & Srivinasan (1986), Bolter & Laing (1983) and Ooi (1986). According to Harcourt (1986), in Ontario, Canada, D. insulare and M. plutellae development coincides with the development of DBM larvae. However D. insulare has better searching capacity, higher fecundity and can avoid superparasitism and multiple parasitism of larvae parasitized by M. plutellae. D. insulae is the most common of the two around many cabbage fields (Bolter & Laing 1983, Harcourt 1986).

Pesticides can have a major effect on biological control. Parasitoids and many predators cannot survive very strong selection pressure from frequent insecticide applications. The greater susceptibility of parasitoids and predators compared to their hosts is most likely due to a difference in the ability of parasitoids and their hosts to detoxify insecticides. Parasites are ecological specialists, having only to attack their host, while the latter have to detoxify numerous toxic components of plants on which they feed (Krieger et al. 1971).

However, exceptions occur. A. plutellae shows slight resistance and still having a high female to male ratio if pesticides are used judiciously (Ooi 1986). In Indonesia, D. eucerophaga is well adapted to an environment with

frequent insecticide applications (Santoso 1979). Parasitism by D. eucerothaga on DBM larvae is not adversely affected by insecticide treatment (Iman et al. 1986). In cotton Microplitis demolitor (Wilkinson) and M. croceipes Cresson both parasitize Heliothis zea (Boddie) larvae and are not affected by the insecticides and fungicides applied to control their host (Felton & Dahlman 1984, Powel et al. 1986, Culin & Dubose 1987). These two parasitoids sometimes have higher parasitism rates in the fields treated with pyrethroids than with organophosphate insecticides.

Fungicides for control of plant pathogens may also affect entomophagous fungi. However, chlorothalonil is widely used for controlling diseases of cabbage, broccoli and other cruciferous crops and is reported not to kill the entomophthoracea fungi, Erynia bluckii (Lakon) and Zoophthora radicans (Brefeld) that attack DBM larvae and pupae especially during wet seasons (Tomiya & Aoki 1982, Wilding 1986). The entomophagous fungus, Verticillium lecani (Zimm) that often affects aphids in potatoes, alfalfa and other cereal crops is highly sensitive to fungicides like metylaxyl, captafol and mancozeb but not to copper or chlorothalonil (Grossman 1990). The parasitoid M. demolitor is also reported to be killed by maneb even at very low concentrations that have no effect on its host H. zea (Powell et al. 1987).

An integration of various techniques, especially in relation to the level and time of pesticides applied in the field with D. insulare and probably other natural enemies of DBM or other pests of cabbage, should be considered and studied extensively. This is particularly important for cabbage growers in the state of Michigan and the United States as a whole, who still overrely on pesticides for control of DBM (Royer et al. 1985, Ghidui 1986, Shelton & Wisely 1986, Linduska 1986, Story et al. 1987).

The objective of this study was to examine the impact of various pesticides normally used in cabbage fields for DBM control and also their effects on its biological control agent, D. insulare. My goal is to develop management systems that favor D. insulare in integrated DBM management in cabbage fields.

Chapter 1

Differential toxicity of pesticides to Diadegma insulare
(Cresson) (Hymenoptera: Ichneumonidae) and its host,
the diamondback moth, Plutella xylostella (L.)
(Lepidoptera: Plutellidae)

ABSTRACT: Studies were done in the laboratory on the differential toxicity of pesticides to adult Diadegma insulare (Cresson) and its host, the diamondback moth (DBM), Plutella xylostella (L.). Leaf dip and direct dip bioassays (DBM larvae) and residual (adult DBM and D. insulare) bioassays were used. None of the pesticides caused 100% mortality to DBM larvae or adults at 1.0 mg active ingredient (AI)/ml concentrations (except Bacillus thuringiensis Berliner var. kurstaki (Bt) and esfenvalerate on DBM larvae and adults, respectively). However, at 1.0 mg AI/ml, all insecticides except B. thuringiensis were extremely toxic to D. insulare, even within 30 min after treatment. Chlorothalonil was not toxic to DBM larvae or adults or to D. insulare. DBM larvae were more sensitive to most insecticides in the direct dip bioassay than in the leaf dip bioassay. Methomyl was the least toxic insecticide to DBM larvae and adults but still caused 100% mortality of D. insulare, 24 h after being exposed (1.0 mg AI/ml). An integrated approach for control of DBM using B. thuringiensis and D. insulare may control DBM without directly affecting D. insulare. The use of chlorothalonil for controlling crop diseases is compatible with DBM management because it has no impact on D. insulare, although it may affect entomopathogenic fungi that attack DBM larvae or adults in the field.

Key words: Insecta, Plutella xylostella, Diadegma insulare, pesticides, toxicity, biological control.

The diamondback moth (DBM), Plutella xylostella (L.), was first recorded as an important pest of cabbage in 1746 (Harcourt 1962). It is a cosmopolitan pest of brassica crops and the primary pest of cabbage in Southern Texas (Cartwright et al. 1987). In general, the level of infestation varies considerably with locality, conducive environments, length of growing season, acres of cruciferous crop grown and frequency of insecticide application (Sun et al. 1978).

Most farmers use conventional chemical pesticides to control DBM. Over-reliance on pesticides has resulted in insecticide resistance, environmental pollution and health hazards (Ho 1965, Georgiou 1981, Miyata et al. 1982, Ooi 1986). Cross-resistance and multiple resistance to insecticides by DBM has also been reported (Liu et al. 1982 and Cheng 1988). To overcome these problems, the microbial insecticide, Bacillus thuringiensis Berliner var. kurstaki (Bt) has been used. However, resistance to Bt has also been reported recently (Tabashnik et al. 1990).

In spite of the wide adaptability of the DBM to new environments and insecticides, it is held below economic thresholds in some regions by natural enemies. Marsh (1917), outlining the DBM situation in the United States, stated that it was suppressed naturally by parasitoids. In southern Ontario, Canada, the major parasitoids of DBM are Diadegma insulare (Cresson), Diadromus subtilicornis (Gravenhorst) (Hymenoptera: Ichneumonidae) and Microplitis plutellae (Muesbeck) (Hymenoptera: Braconidae) (Harcourt 1960). D. insulare is more competitive than the other two species (Bolter & Laing 1983). Consequently, D. insulare is the most abundant and important parasitoid of DBM in southern Ontario, Canada (Harcourt 1960). D. insulare development is highly synchronized with the development of its host. It has excellent searching capacity, and has four or five generations per year. It

overwinters as a pupa, within the pupa of its host or among the remnants of the crop (Harcourt 1960). These characters could be manipulated for DBM management (Harcourt 1986). D. insulare's parasitism activity increases at a decreasing rate as its population increases and is at times reinforced by that of M. plutellae and D. subtilicornis (Harcourt 1969 and 1986). D. insulare parasitizes the first three instars and kills its host in the prepupa stage (Bolter & Laing 1983) and has been reported to cause larval mortality as high as 75% (Harcourt 1969).

In Indonesia, Diadegma eucerophaga (Horstmann) (Hymenoptera: Ichneumonidae) kills between 60% and 90% of DBM larvae (Sastrosiswojo & Sastrodiharjo 1986). D. eucerophaga and Apanteles plutella (Kurdjumov) (Hymenoptera: Braconidae) also appear to be well adapted to an environment frequently treated with insecticides (Santoso 1979, Chua & Ooi 1986).

High rates of host mortality achieved by D. insulare and other DBM parasitoids indicate a potential for using them as an alternative method for controlling DBM in cabbage fields. However, pesticides can have a detrimental impact on D. insulare. For the foreseeable future, insecticides will remain as a powerful and essential tool in DBM management, but their use must be minimized to prevent a trend of over-reliance on pesticides and resistance development. An approach that could integrate the use of pesticides and D. insulare to control DBM should be developed.

The objective of this study was to examine the differential impact of synthetic pyrethroid, organophosphate, carbamate and microbial insecticides and the fungicide, chlorothalonil, on an insecticide-resistant strain of DBM larvae and adults, and on D. insulare adults.

Materials and Methods

Field Collection. DBM larvae were collected from infested commercial cabbage fields in Monroe County, Michigan during October 1989. Growers in this area had reported control failures after applications of insecticides, including methomyl (carbamate), permethrin and esfenvalerate (pyrethroids) and azinphosmethyl (organophosphate). Cabbage plants were randomly selected from the infested fields, cut at the roots, and brought back to the laboratory in plastic bags. Parasitized and non-parasitized pupae from the fields were kept separately in petri dishes at 5°C. (Parasitized pupae are 50% shorter than non-parasitized pupae, a little smaller in diameter, oblong in shape, the developing eye cannot be seen externally as it can on non-parasitized DBM pupae, and there is a small black or dark brown spot at one end of the thick, silk pupae).

DBM larvae from the field were fed untreated fresh broccoli leaves every two days and held at room temperature ($21 \pm 2^\circ\text{C}$) and 16:8 (L:D) photoperiod until pupation. Parasitized and non-parasitized pupae were separated as before and kept at 5°C for future rearing.

DBM rearing. A 200 ml plastic cup nearly filled with water was used to hold broccoli leaves. Holes were made in the cup cover and stems of five to six young broccoli leaves were inserted into the cup through the hole. The cup and the leaves were placed inside a 45 x 35 x 35 cm ovipositional cage ($21 \pm 2^\circ\text{C}$, 16:8 (L:D) photoperiod). DBM pupae were then placed inside the cage for adult emergence. A muslin cloth wetted with diluted honey was put in a small petri dish inside the cage to feed the adult DBM. A few drops of diluted honey were added to the muslin cloth every two days. After five to seven days the infested leaves with eggs and first and second instar DBM were

transferred to a 30 x 20 cm x 15 cm deep cake pan, with a 6 x 6 cm screened opening in the lid, for further rearing. These larvae were fed fresh broccoli leaves every two days until pupation.

D. insulare rearing. Greenhouse-reared broccoli plants were transferred to rearing cages (60 x 45 x 45 cm) ($21 \pm 2^{\circ}\text{C}$, 16:8 (L:D) photoperiod). They were inoculated with 2nd or 3rd instar DBM and D. insulare pupae. D. insulare adults which emerged from the pupae were fed with honey as above. Leaves with 3rd to 4th instar DBM were harvested about five days later and the larvae were transferred to a modified cake pan until they pupated. D. insulare pupae were collected for further rearing or experiments.

Pesticides Used. Insecticides used were commercial formulations of permethrin (Ambush 2 EC), azinphosmethyl (Guthion 3 F), methomyl (Lannate 1.8 L), esfenvalerate (Asana XL 0.66 EC), cypermethrin (Ammo 2.5 EC), Bacillus thuringiensis Berliner var. kurstaki (Dipel 2X) and the fungicide chlorothalonil (Bravo 720). They represent the most commonly recommended pesticides in Michigan for control of insect pests and diseases of cabbage, broccoli and other cruciferous crops.

Bioassays

DBM larvae. Leaf dip bioassays were carried out to determine the response of DBM larvae (9th generation of field collection, cultured without insecticide selection) to ingestion of the pesticides listed above. Leaves (small enough to fit in a 14.5 cm diameter plastic petri dish) were cut from frame leaves of uninfested broccoli raised in the greenhouse. Individual leaves were randomly selected, immersed in a prepared pesticide solution (100, 10, 1.0, 0.5, 0.1 or 0.01 mg active ingredient (AI)/ml distilled water) for three seconds and air dried for 2 h. Control leaves were treated similarly with tap

water. A 10 cm diameter filter paper (Whatman No. 1) was placed inside a 14.5 cm diameter petri dish. The treated leaves were placed on top of the filter paper (one per dish) and randomly-selected third instar DBM were released in each petri dish and held at $21 \pm 2^{\circ}\text{C}$ and 16:8 (L:D) photoperiod. The treatments were replicated four times (four petri dishes for each concentration) and larval mortality was recorded at 8, 16, 24, 48 and 72 h. Larvae were considered dead if they could not walk one body length when gently probed.

Mortality on the treated leaves was corrected for mortality on untreated leaves using Abbott's (1925) formula. Data was analyzed using probit analysis and analysis of variance (MSTAT, Eisensmith & Russel 1989). LC_{50} values were statistically compared using overlap or non-overlap of 95% confidence limits.

Direct dipping of larvae into the pesticide solution was used to evaluate contact toxicity. Five randomly-selected 3rd instar DBM were placed in a 4 cm diameter tea strainer and dipped in prepared pesticide solutions (100, 10, 1.0, 0.5, 0.1 or 0.01 mg AI/ml tap water) for three seconds. Larvae then were removed from the tea strainer, blotted dry on paper towel and released on untreated broccoli leaves in 10 cm diameter petri dishes as before. Concentrations were replicated four times and mortality was recorded and analyzed as above.

DBM adults. A residual bioassay was done primarily to enable us to compare the response of DBM and D. insulare adults where both species might be walking on treated surfaces in the field. Pesticide solutions were prepared as in the earlier bioassays. The prepared test solutions were poured into test tubes (18 cm x 15 mm) and swirled around for one minute. The excess was poured off and the residue was air dried for 2 h. The

concentrations were replicated four times (four test tubes each). Five randomly-selected DBM adults (less than one week old) were released in each of the treated test tubes. The top of the test tube was closed with a cotton ball. Diluted honey was supplied as food through the cotton plug. The insects were held at $21 \pm 2^\circ\text{C}$ and 16:8 (L:D) photoperiod. Mortality was recorded after 8, 16, 24, 48 or 72 h by gently shaking each test tube a few times, holding it horizontally for 10 seconds and returning it back upright. Insects that fell to the bottom of the test tube and were unable to climb up the sides were considered dead. Data were analyzed as for earlier bioassays.

D. insulare. Treated test tubes were prepared as for the DBM adult bioassay. Five randomly selected D. insulare adults were released into each treated test tube and held at $21 \pm 2^\circ\text{C}$ and 16:8 (L:D) photoperiod. Determination of mortality was similar to DBM adult bioassay. However, mortality was recorded at 30 minutes and 24 h.

Results and Discussion

DBM larvae. Mortality of DBM larvae at 1.0 mg (AI)/ml concentrations in the leaf dip bioassay was significantly higher with permethrin, esfenvalerate, cypermethrin, B. thuringiensis (Bt) and azinphosmethyl, than with methomyl or chlorothalonil (Figure 1A, B & C) (at 48 h; Tukey's test, $P < 0.05$, $F = 22.70$, $df = 6, 18$). The LC_{50} value for methomyl was significantly higher than LC_{50} values for the other insecticides (non-overlapping confidence limits) (Table 1). Although the mortality of DBM larvae treated with 1.0 mg AI/ml Bt was not different than larvae treated with 1.0 mg AI/ml permethrin, esfenvalerate, cypermethrin or azinphosmethyl ($P \leq 0.05$, Tukey's test, $F = 23.81$, $df = 6, 18$) (Figure 1); its LC_{50} value was significantly lower 48 h

Table 1. Response levels and LC50 values with 95% confidence limits, Chi-square and slope $\pm$ SE for all pesticides at 24 or 48 hours (Diamondback moth adults and larvae) and 30 minutes or 24 hours (*Diadegma insulare*) after treatment.

Pesticide ^a	Diamondback Moth				<i>D. insulare</i> (residual bioassay)	
	larva (48 h)		adults (24 h)		30 min	24 h
	Leaf dip	direct dip	residual			
permethrin						
LC50(n)	0.135(120)	0.005(120)	0.306(120)		0.490(120)	<0.01(120) ^b
95% C.L.	0.074 - 0.197	0.0003 - 0.088	0.294 - 0.320		0.445 - 0.541	--
slope $\pm$ SE	0.813 $\pm$ 0.122	0.510 $\pm$ 0.111	1.304 $\pm$ 0.092		1.802 $\pm$ 0.296	--
chi-square(df) ^c	1.073(4)	0.908(4)	1.253(4)		0.411(4)	--
cypmethrin						
LC50(n)	0.265(120)	0.082(120)	1.006(120)		0.076(120)	<0.01(120) ^b
95% C.L.	0.217 - 0.323	0.060 - 0.112	0.978 - 1.035		0.067 - 0.086	--
slope $\pm$ SE	1.190 $\pm$ 0.323	0.955 $\pm$ 0.141	1.714 $\pm$ 0.132		1.929 - 0.280	--
chi-square(df) ^c	1.001	1.505	0.521(4)		1.631(4)	--
esfenvalerate						
LC50(n)	0.195(120)	0.116(120)	0.0515(120)		<0.01(120) ^b	<0.01(120) ^b
95% C.L.	0.146 - 0.268	0.086 - 0.158	0.049 - 0.053		--	--
slope $\pm$ SE	0.895 $\pm$ 0.131	0.899 $\pm$ 0.129	1.181 $\pm$ 0.135		--	--
chi-square(df) ^c	1.380(4)	0.458(4)	0.292(4)		--	--
azinphosmethyl						
LC50(n)	0.214(120)	0.004(120)	0.457(120)		0.645(120)	<0.01(120) ^b
95% C.L.	0.177 - 0.258	0.0002 - 0.105	0.347 - 0.602		0.581 - 0.716	--
slope $\pm$ SE	1.240 $\pm$ 0.201	0.499 $\pm$ 0.112	1.601 $\pm$ 0.125		1.738 $\pm$ 0.279	--
chi-square(df) ^c	0.423(4)	0.875(4)	1.339(4)		0.280(4)	--

Table 1 continued

Pesticide ^a	Diamondback Moth				<u>D. insulare</u> (residual bioassay)	
	larva		adults		30 min	24 h
	Leaf dip	direct dip	residual			
<u>methomyl</u>						
LC50(n)	1.726(120)	8.275(120)	3.890(120)		4.324(120)	0.173(120)
95% C.L.	1.384 - 2.153	5.556 - 12.177	2.307 - 5.374		3.613 - 5.177	0.169 - 0.179
slope $\pm$ SE	1.100 $\pm$ 0.013	0.818 $\pm$ 0.113	1.567 $\pm$ 0.113		1.367 $\pm$ 0.178	3.031 $\pm$ 0.254
chi-square(df) ^c	0.476(4)	2.396(4)	0.999(4)		0.557(4)	0.484(4)
<u>B. thuringiensis</u>						
LC50(n)	0.014(120)	>100.0(120) ^d	>100.0(120) ^e		>100.0(120) ^b	>100.0(120) ^e
95% C.L.	0.009 - 0.020	--	--		--	--
slope $\pm$ SE	0.931 $\pm$ 0.140	--	--		--	--
chi-square(df) ^c	0.904(4)	--	--		--	--
<u>chlorothalonil</u>						
LC50(n)	>100.00(120)	9.733(120)	>100.00(120) ^e		>100.0(120)	>100.0(120) ^e
95% C.L.	--	6.194 - 15.293	--		--	--
slope $\pm$ SE	--	0.770 $\pm$ 0.109	--		--	--
chi-square(df) ^c	--	2.652(4)	--		--	--

a, LC50 = mg AI/ml; b, all killed after 30 minutes at 0.01 mg AI/ml; c, $\bar{P} < 0.05$; d, 15% mortality at 100 mg AI/ml and e, 5% mortality at 100 mg AI/ml.

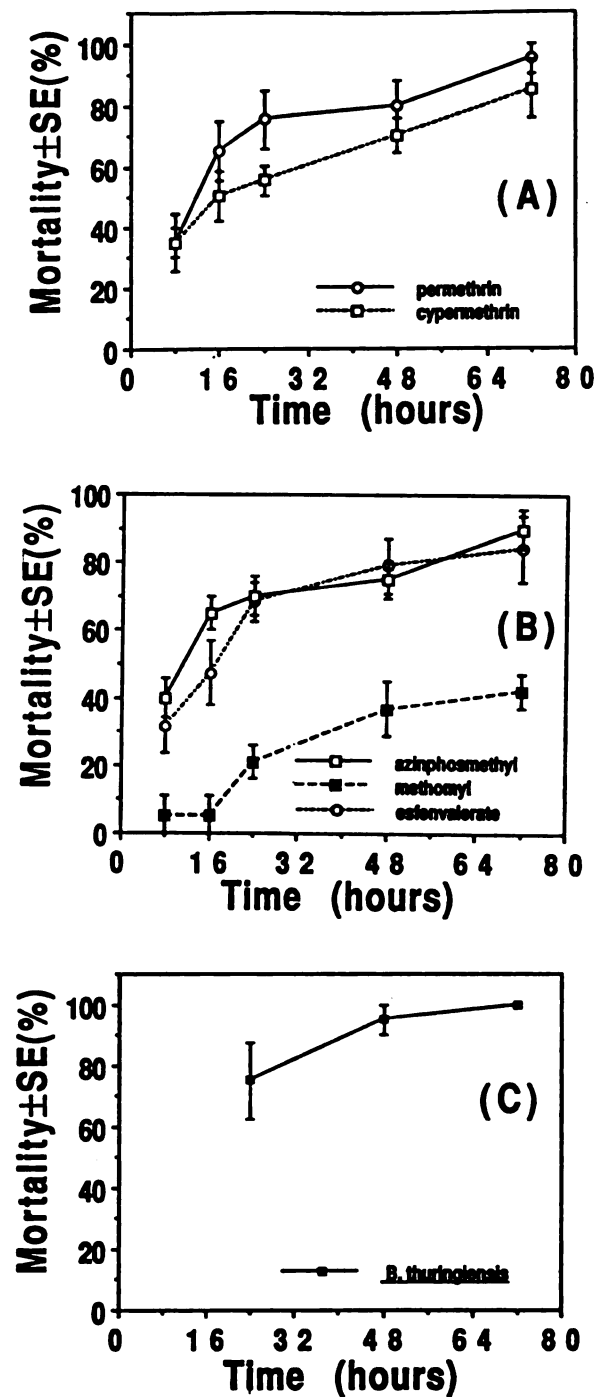


Figure 1. Percent mortality ($\pm$ SE) of diamondback moth larvae at 8, 16, 24, 48 and 72 hours after being exposed to 1.0 mg (AI)/ml pesticide solution in leaf dip bioassay.

after treatment (Table 1). Some larvae were observed avoiding eating insecticide-treated leaves indicating behavioral resistance exists among the population. The fungicide chlorothalonil did not kill DBM larvae at any concentration in the leaf dip assay.

Magaro and Edelson (1990) reported the same trend using a leaf dip method to test permethrin, esfenvalerate and methomyl at recommended field rates (65, 64 and 15% mortality for permethrin, esfenvalerate and methomyl, respectively). None of the pesticides in our study caused 100% mortality to DBM larvae at 1.0 mg (AI)/ml (Figure 1A, B and C). The 95% mortality (48 h) caused by 1.0 mg (AI)/ml of Bt (Figure 1C) was comparable to what Tabashnik et al. (1990) reported. In their study, at 48 h after treatment, mortality from Bt was 90-100%, 60-90% and 34-35% for susceptible laboratory strains, minimally treated field DBM populations and resistant field populations, respectively. Therefore, the DBM larvae collected from Monroe County, Michigan were highly susceptible to Bt treatment.

Larvae tested in this experiment were the 9th generation after field collection, and were cultured in laboratory without insecticide selection. Mortality could be even lower if the larvae tested were collected directly from the field, since susceptibility of the larvae to pesticides generally increases as the number of generations from the field increases (Iman et al. 1986, Sun et al. 1986).

In the direct dip bioassay, there were no significant differences between larval mortality caused by pyrethroids or azinphosmethyl (Figure 2A, B, & C) (at 48 h, Tukey's test, $P > 0.05$, $F = 68.56$, $df = 6, 18$). Chlorothalonil caused higher mortality when larvae were dipped in test solutions than when leaves were dipped. There was no larval mortality with Bt because Bt requires ingestion

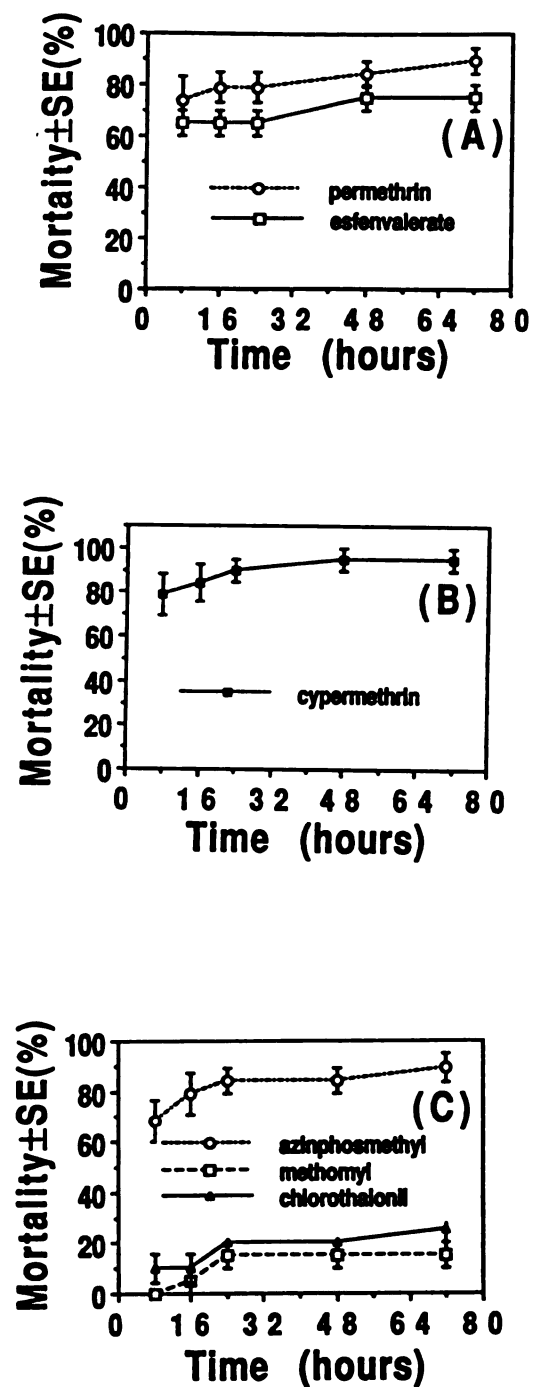


Figure 2. Percent mortality ($\pm$ SE) of diamondback moth larvae at 8, 16, 24, 48 and 72 hours after being exposed to 1.0 mg (AI)/ml pesticide solution in direct dip bioassay.

(Fast & Donaghue 1971). Methomyl was the least toxic chemical insecticide and its LC_{50} was significantly higher than the others (Table 1).

Mortality of DBM larvae in the direct dip bioassay was higher than in the leaf dip bioassay for the pyrethroids (permethrin, esfenvalerate and cypermethrin) and the organophosphate (azinphosmethyl) (Figures 1 & 2). Methomyl caused very low mortality in both bioassays.

The LC_{50} values for permethrin, cypermethrin, esfenvalerate and azinphosmethyl were not significantly different and all were significantly lower than methomyl (Table 1). This indicated that, by contact action, methomyl was still the least effective insecticide for controlling DBM larvae, although factors such as residual activity also affect field results.

DBM adults. Esfenvalerate caused 100% mortality to DBM adults in the residual bioassay 16 h after being exposed to 1.0 mg (AI)/ml concentration (Figure 3A). This mortality was significantly higher than mortality caused by any of the other pesticides (Tukey's test, $P < 0.05$, $F = 47.30$, $df = 6, 18$) (Figures 3A & B). At 24 h after treatment, the LC_{50} for esfenvalerate was significantly lower than for the other pesticides (Table 1). DBM adults were not affected by either chlorothalonil or Bt, and only 15% were killed by methomyl at 1.0 mg (AI)/ml (24 h), and mortality by these three pesticides did not differ significantly. Higher sensitivity to esfenvalerate than to other pesticides by DBM adults is also reported by Iman et al. (1986), Sun et al. (1986), Tabashnik et al. (1987), and Cheng (1988). Of all pyrethroids that are commonly used for DBM control, esfenvalerate loses its effectiveness soonest after frequent application (Tabashnik et al. 1987 and Cheng 1988). However, effectiveness of permethrin, cypermethrin and azinphosmethyl were similar to each other, as they were to DBM larvae in leaf and direct dip bioassay (Figures 1, 2 & 3).

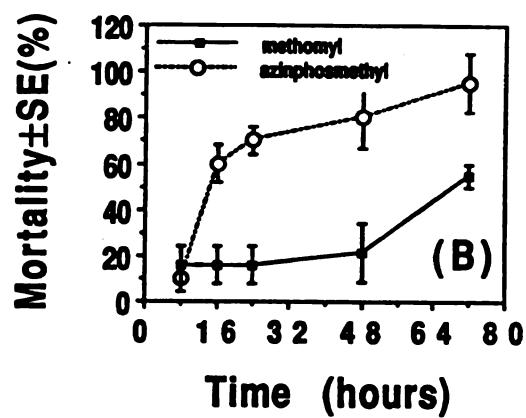
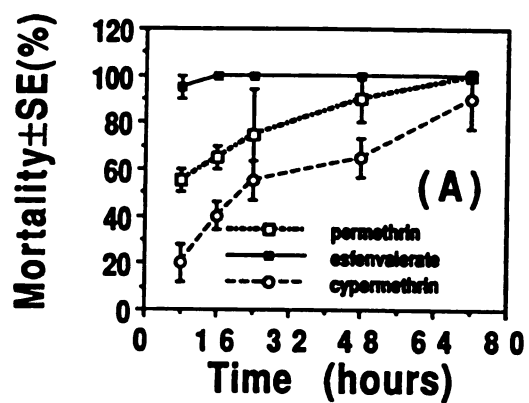


Figure 3. Percent mortality ($\pm$ SE) of diamondback moth adults at 8, 16, 24, 48 and 72 hours after being exposed to 1.0 mg (AI)/ml pesticide solution in residual bioassay.

The DBM population that we studied appeared to be heterozygous with respect to insecticide resistance. Slopes of the probit mortality regressions were usually low (≤ 1.0) and chi-square values were often >1.0 , indicating high variability.

Little is known about the impact of pesticide residues on DBM adults. In the field, adult DBM are often exposed to insecticides applied to control larvae. The effects of insecticides on mobile adults has substantial impact on dispersal and population dynamics of DBM (Tabashnik et al. 1987, 1988). Ruscoe (1977) found that in the field, permethrin residues on cabbage leaves were less toxic to DBM adults than to larvae. In addition, DBM adults show behavioral responses to insecticides by automizing their metathoracic legs at the trochanter-femur joint after walking on surfaces coated with pyrethroid insecticide residues (Chen et al. 1985, Tabashnik 1988). Moths recover from knockdown, shed their affected legs and fly normally though having reduced fecundity (Moore & Tabashnik 1989). This behavioral response is heritable and will contribute to future problems in DBM management (Moore & Tabashnik 1989).

Diadegma insulare. All chemical insecticides (including methomyl, which was low in toxicity to DBM larvae and adults) were highly toxic to D. insulare, even within 30 minutes after treatment (Figures 4A & B). Like DBM adults, D. insulare were more sensitive (after 30 minutes) to esfenvalerate than to azinphosmethyl, methomyl, Bt or chlorothalonil (Figure 4A).

The LC_{50} value for esfenvalerate at 30 minutes also was lower (<0.01) than any for the other insecticides (Table 1). Methomyl was significantly less toxic after 30 minutes to D. insulare than were the other chemical insecticides. Iman et al. (1986) reported that, after 1 h for D. eucerotheca exposed to treated test tubes, the carbamate (carbaryl) was 65 times less toxic than esfenvalerate.

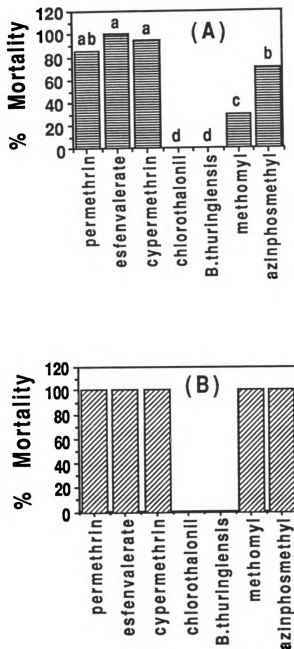


Figure 4. Mortality of *Diadegma insulare* at 30 minutes (A) and 24 hours (B) after being exposed to a 1.0 mg (AI)/ml pesticide solution in a residual bioassay. Bars with the same letter are not significantly different [Tukey's test (A) at $P > 0.05$, $F = 106.6$ and $n = 28$ replication of 5 adults *D. insulare*]. Data in B were either 100% or 0% and therefore could not be analyzed using standard ANOVA.

At 24 h methomyl (carbamate) was 22.5 times less toxic to DBM than to D. insulare (LC₅₀ ratio = 3.89 for DBM adults/0.173 for D. insulare). LC₅₀ values for D. insulare for the other insecticides are <0.01 mg (AI)/ml (Table 1) and the selectivity ratios for DBM vs. D. insulare are greater than 30.6, 100.0, 5.1 and 45.7 for permethrin, cypermethrin, esfenvalerate and azinphosmethyl, respectively.

Neither chlorothalonil nor Bt caused mortality to D. insulare adults at any concentration. The braconid parasitoid, A. plutellae, was also reported to be unaffected in the field after treatment with Bt (Sivapragesan et al. 1987), and Bt had no effect on the parasitism of spruce budworm larvae (Niwa et al. 1987). However, Santoso (1979) reported that 7 d after treatment (laboratory studies), Bt residues on treated leaves caused 20% mortality of D. eucerothaga adults and 100% to DBM larvae.

The high sensitivity to chemical insecticides that D. insulare shows in this experiment is supported by Iman et al. (1986). In laboratory experiments (residual bioassay on the parasitoids and DBM, at 1 h after treatment) pyrethroid insecticides (permethrin, cypermethrin and esfenvalerate) were more toxic to D. eucerothaga than to DBM. The selectivity ratio (DBM LC₅₀/D. eucerothaga LC₅₀) were 12.2, 19.7, 39.2 and 8.7 for permethrin, cypermethrin, esfenvalerate and carbaryl respectively. It appears that D. insulare is more sensitive than D. eucerothaga to chemical insecticides.

In contrast to D. insulare, Microplitis croceipes (Cresson) (Hymenoptera: Braconidae) adults, a parasitoid of the cotton bollworm, Heliothis zea (Boddie) (Lepidoptera: Noctuidae) and tobacco budworm, Heliothis virescens (Fabricius) (Lepidoptera: Noctuidae) is somewhat tolerant of pyrethroid insecticides (Powel et al. 1986). Croft and Whalon (1982) also reported that certain hymenopteran parasitoids are tolerant of pyrethroid

materials. D. eucerophaga in Indonesia seem to be well adapted to an environment increasingly treated with pesticides (Santoso 1979). Therefore, D. insulare may become tolerant as was shown for D. eucerophaga after repeated exposure to insecticides.

Chlorothalonil was expected not to kill DBM, but is used heavily for controlling diseases of cruciferous crops and might have had an effect on D. insulare. My results indicate that it is not toxic to D. insulare. However, Felton and Dahlman (1984) reported that the fungicide, maneb, is more toxic to M. croceipes than to its host, H. virescens, even at very low concentrations. Chlorothalonil is also reported not to kill the Entomophthoraceae fungi, Erynia bluckii (Lakon) and Zoophthora radicans (Brefeld) that often infect DBM larvae and pupae (Tomiyama & Aoki 1982 and Wilding 1986). Entomophthora fungi, Verticillium lecani (Zimm.) Viegas that often affect aphids in potatoes, alfalfa and other crops are killed by the fungicides metylaxyl, captafol and mancozeb, but not by copper or chlorothalonil (Grossman 1990).

Conclusion

Based on these results and also on previous reports it is clearly indicated that we need an integrated approach to control DBM. D. insulare is not compatible with the chemical insecticides that we tested. These results indicated that esfenvalerate is the most toxic pyrethroid for DBM control, but it would eliminate most of the D. insulare. Bt, however, was the safest pesticide to use for DBM control because it did not cause parasitoid mortality even at a concentration of 100 mg (AI)/ml. Bt did not appear capable of

killing all the DBM larvae in this study except at 100 mg (AI)/ml, although it was the safest pesticide for D. insulare.

Conversely, D. insulare alone is probably incapable of controlling and regulating the DBM population at a level that will not cause economic damage to cruciferous crops. Parasitism of DBM larvae generally ranges from 25-75% (Harcourt 1969). High D. insulare populations in the field may be self-limiting because the parasitism rate decreases as the parasitoid population increases, due to mutual interference between searching females (Putnam 1968). Therefore, insecticides are still necessary to manage DBM. DBM larvae may be best controlled by using Bt with D. insulare. Larvae that escaped or survived from D. insulare attack could be controlled by Bt. By integrating the use of D. insulare and Bt to control DBM we could prolong the efficacy of Bt in the field and slow down the development of resistance by DBM to Bt (Tabashnik et al. 1990).

Many studies have been done on the effectiveness of insecticide mixtures (Bt with other chemical insecticides) on DBM larvae (Iman et al. 1986, Yeh et al. 1986). Iman et al. (1986) reported that a mixture of Bt and esfenvalerate (16.0 kg (AI)/ha + 0.04 kg (AI)/ha) was more effective than treatment with one of these insecticides alone. Parasitism of DBM larvae by D. eucerotheca was not adversely affected by this mixture. No such study was done on D. insulare. It may be possible to use mixtures of insecticides with Bt in controlling DBM without affecting D. insulare greatly. The laboratory bioassay chiefly tested the physiological or metabolic resistance that may inhibit behavioral responses of D. insulare toward pesticides when applied in the field. However, more in-depth studies have to be carried out. Also, insecticide mixtures may not be advisable for management of insecticide

resistance. Insecticide mixtures should give greater control of DBM but still be compatible with D. insulare.

We recommend the use of chlorothalonil for controlling fungal diseases. It does not appear to kill D. insulare and may also be compatible with entomopathogenic fungi that often attack larvae and pupae of DBM (Wilding 1986). The integration of D. insulare with Bt and chlorothalonil may be the best integrated and most stable system for DBM management.

Chapter 2

Field studies on the impact of pesticides on the
diamondback moth, Plutella xylostella (L.) (Lepidoptera:
Plutellidae) and parasitism by Diadegma insulare (Cresson)
(Hymenoptera: Ichneumonidae)

ABSTRACT: Parasitism of DBM was surveyed and the impact of pesticides on the diamondback moth (DBM), Plutella xylostella (L.) and parasitism by Diadegma insulare (Cresson) was studied at the Michigan State University Research Farm during July and August, 1990. Pesticides used were Bacillus thuringiensis (Bt), thiodicarb, thiodicarb + Bt, chlorpyrifos, permethrin, ICIA 0321 (pyrethroid) and esfenvalerate. DBM larval mortality was not different among the treated plots but was significantly higher than in untreated plots 3 d after spraying. The change in number of DBM larvae (0 vs 3 d), DBM and D. insulare pupae (0 vs 6 d) was also not significant between the treatments. Bt applied at 1.8 kg/ha significantly reduced numbers of D. insulare pupae more than Bt applied at 1.4 kg/ha. Parasitism by D. insulare of the DBM larvae in treated rows was not seriously affected 6 d after insecticide spraying. Mean change in percent of parasitism by D. insulare on DBM larvae in guard rows was also not different. However, the percent parasitism in cages treated with permethrin, azinphosmethyl or Bt and untreated were 7.8%, 13.3%, 81.5% and 79.4%, respectively. The range of DBM parasitism from three different locations in Michigan was 60.8-78.9%. Parasitism by D. insulare of the DBM larvae seems to be influenced by the vegetation or refuge plants present outside of the experimental plots. DBM parasitism by D. insulare may not be severely affected if insecticide spraying is carried out judiciously.

Key words: Insecta, diamondback moth (DBM), D. insulare, parasitism.

The diamondback moth (DBM), Plutella xylostella (L.), is a major worldwide pest of cabbage and other crucifers (Talekar et al. 1986). Most cabbage growers use insecticides to control DBM which constitute about 30% of the production costs (Lim 1972). The failure to control DBM in crops and the species' resistance to insecticides (including Bacillus thuringiensis Berliner var. kurstaki have been reported by Sun et al. (1978), Liu et al. (1982), Georghiou (1981), Chen & Sun (1986), Magaro & Edelson (1990) and Tabashnik et al. (1990). Resistance problems mainly resulted from excessive and frequent use of insecticides (Magaro & Edelson 1990, Cheng 1988, Chua & Ooi 1986), as well as large acreage and continuous planting seasons (Sun et al. 1978).

DBM is capable of developing resistance to all groups of insecticides even after only a few applications (Sun et al. 1986, Yeh et al. 1986). Cross-resistance and multiple resistance is common (Cheng 1988, Takeda et al. 1986). Resistance ability is due to different microsomal functional oxidase enzymes in DBM larvae (Cheng 1988), as well as physiological and behavioral responses of both DBM larvae and adults (Tabashnik et al. 1987, Moore & Tabashnik 1989). DBM is reported to develop resistance faster to more toxic insecticides like pyrethroids, as opposed to less toxic insecticides such as carbamate (Cheng 1988).

Historically, DBM was held below economic thresholds in the U.S.A. by natural enemies (Marsh 1917). In southern Ontario, Canada, Diadegma insulare (Cresson) (Hymenoptera: Ichneumonidae) is a major specific parasitoid of DBM (Harcourt 1960, 1963) and parasitizes up to 75% of DBM larvae (Harcourt 1969 & 1986, Bolter & Laing 1983, Lasota & Kok 1986). In Indonesia, Diadegma eucrophaga (Horstmann) kills approximately 85% of the DBM larval population (Sastrosiswojo & Sastrodiharjo 1986). Diadromus

subtilicornis (Gravenhorst) (Hymenoptera: Ichneumonidae) and Microplitis plutellae (Muesbeck) (Hymenoptera: Braconidae) also attack DBM, however D. insulare's high synchronization with its host developmental stage and excellent searching capacity make it more suitable for integrated DBM management (Harcourt 1969, 1986). D. eucerotheca and another DBM parasitoid, Apanteles plutellae (Kurdjumov) (Hymenoptera: Braconidae) are reported to be well adapted to an environment frequently treated with insecticides (Ooi 1986, Iman et al. 1986, Sastrosiswojo & Sastrodiharjo 1986). High rates of host mortality achieved by D. insulare and other DBM parasitoids indicate that they may be used as an alternative or supplemental method for controlling DBM in the field.

Insecticides will remain a major tool in DBM management in the near future. However, their use must be minimized to slow insecticide resistance development by DBM. An approach that could integrate the use of pesticides and D. insulare to control DBM should be developed, however, most pesticides can have a detrimental impact on D. insulare. Laboratory studies (Chapter 1) showed that all chemical insecticides were extremely toxic to adult D. insulare even at very low concentrations that caused no mortality to DBM adults.

The objective of this study was to assess general levels and species of DBM parasitoids in Michigan and to evaluate the effect of insecticides in the field on the mortality of DBM larvae and parasitism by D. insulare.

Materials and Methods

Parasitism of DBM in Michigan. DBM larvae (2nd, 3rd and 4th instar) and the pupae of DBM and D. insulare were collected from the Michigan State

University (MSU) Entomology Research Farm, from a greenhouse in Eaton County and a commercial cabbage field in Monroe County. The broccoli plants from the MSU Research Farm were from Eaton County. All DBM larva from Eaton County were collected before transplanting. This was to ensure that parasitized DBM larvae collected from the MSU Research Farm were not mixed with those from Eaton County.

DBM larvae were brought back and reared in the laboratory until pupation. The number of DBM and parasitoid pupae formed were recorded and the percent of D. insulare parasitism calculated as the total number of D. insulare pupae divided by the total number of D. insulare and DBM pupae x 100.

The adults emerging from parasitoid pupae at the MSU research farm were sent for identification to the Taxonomy Services Unit, Systematic Entomology, Plant Sciences Institute, Room 101A, Building 046, Beltsville Agriculture Research Center-West, Beltsville, MD 20705. I am indebted to R.W. Carlson for parasitoid identifications.

Field Plot Experiment. Broccoli plants were transplanted into the field at the MSU Entomology Research Farm on July 5, 1990. Rows were 0.9 m apart and plants were 0.6 m apart in the row. Plots were 1 row x 7.6 m long, separated by an untreated row and there were untreated border rows on each side of the plot. Treatments were arranged in a Randomized Complete Block manner with four replications per treatment.

Two thousand DBM adults (2 to 4 days old) were released in the plot on July 10, 1990. These adults were reared in the laboratory from adults and larvae originally collected from the Monroe County field. A preliminary survey (12 plants per plot) was carried out to determine the number of DBM larvae and D. insulare pupae on August 5, 1990. DBM larval density was still

too low (<1.0 per plant) and an additional 3,600–4,000 2nd instar DBM were released on August 8th (approximately four to five larvae per plant). D. insulare pupae were approximately 75% of the total pupae (D. insulare and DBM) collected and there was no need to release additional D. insulare.

DBM larvae and D. insulare pupae were sampled again on August 16th (six plants per plot). All DBM pupae and D. insulare pupae on the six plants were collected two hours before insecticides were sprayed. This ensured that only new pupae formed after spraying were present at the time of sampling. Treatments were applied on August 16th, using a hand-held carbon CO₂ sprayer with one nozzle over the row at 4.5 kg per cm² pressure and 458 l/ha. Pesticides used were experimental or commercial formulations of Bacillus thuringiensis (Bt) or standard chemical insecticides (Table I).

The number of live DBM larvae and also DBM and D. insulare pupae on six plants per plot were counted and recorded 3 d after treatment (for larvae) and 6 d after treatment (for pupae). Data were transformed using $\log(1 + x)$ (Harcourt 1960, Wadley 1950) before standard analysis of variance (MSTAT, Eisensmith & Russell 1989).

Parasitism in guard rows. Ten guard rows in the experimental plot were randomly selected. DBM and D. insulare pupae were sampled approximately 2 h before and 6 d after insecticide treatment of the adjacent treated rows. Sampling was similar to treated rows. The change in the number of DBM and D. insulare pupae as well as the percent parasitism of D. insulare on DBM was analyzed by a paired t-test (MSTAT, Eisensmith & Russell 1989).

Field Cage Experiment. The purpose of this experiment was to obtain more accurate data about the impact of pesticides on the parasitoid if D. insulare were prevented from entering the experimental area after treatment.

Table 1. Pesticides used in the field studies on the impact of pesticides on the diamondback moth and parasitism by Diadegma insulare at the Michigan State University Entomology Research Farm.

Common Name	Trade Name	Additive	Rate
<u>Bacillus thuringiensis</u> Berliner var. <u>kurstaki</u> (Bt)	Javelin 100 DF (Sandoz Crop Protection Corp.)	none	1.4 kg/ha
	"	none	1.8 kg/ha
	CGA 237218 (Ciba-Geigy Agricultural Div., NC)	none	3.7 kg/ha
	"	none	7.4 kg/ha
	MVP aqueous 21848 (Mycogen Corp. San Diego, CA)	none	7.6 kg/ha
	"	Sticker	(7.6+1.1) kg/ha
	"	Sunspray ultrafine oil	7.6 kg/ha + 2.8 l/ha
thiodicarb	Larvin 3.2 (Rhone Poulenc, Raleigh, NC)	none	0.67 kg AI/ha
	"	Javelin 100 DF	0.67 kg AI/ha + 1.8 kg/ha
chlorpyrifos	Lorsban 50 WP (Dow Elanco, Indianapolis, IN)	none	1.12 kg AI/ha
permethrin	Ambush 2E (ICI Americas, Inc. Wilmington, DE)	none	0.11 kg AI/ha
	ICIA 0321 1E (ICI Americas, Inc., Wilmington, DE)	none	0.02 kg AI/ha
esfenvalerate	Asana XL 0.66 EC (Du Pont Agricultural Products, Wilmington, DE)	none	0.03 kg AI/ha

This might simulate a large commercial field with little or no external source of D. insulare. Twelve cages (180 x 165 x 165 cm) were placed randomly along the border rows of the broccoli plots enclosing six individual broccoli plants in each cage. The existing DBM larvae and the pupae of DBM and D. insulare were thoroughly searched and collected from each broccoli plant within the cages. Some flowering weeds (several each) of horseweed, Conyza canadensis (L.), common lambsquarters, Chenopodium album L., black mustard, Brassica nigra L. (Kock) and rough fleabane, Erigeron strigosus Muhl., were transplanted into the cages to serve as food sources for the adult parasitoids. Three days later, twenty 2nd to 3rd instar DBM were released on each broccoli plant and five pairs of three to five-day old D. insulare adults were released in each cage.

Six days later ten 3rd-4th instar DBM were collected from each broccoli plant. They were brought back to the laboratory and reared in a modified cake pan until pupation. Numbers of parasitized and non-parasitized pupae were recorded and analyzed by analysis of variance (MSTAT, Eisensmith & Russell 1989). Immediately after the larvae were collected, permethrin (0.76 l/ha or 0.2 ml/cage), azinphosmethyl (1.4 l/ha or 0.04 ml/cage), Bt (4.0 kg/ha or 1.1 g/cage) or water (control) were sprayed in the respective cages (3 cages = replications per treatment). To assess parasitoid survival, 2nd to 3rd instar DBM (20 per plant) were released 4 d later on each broccoli plant. Twenty 3rd to 4th instar DBM per plant were collected 6 d later and reared in the laboratory until pupation. The numbers of DBM and parasitoid pupae were again recorded and analyzed as before.

Results and Discussion

Parasitism of DBM in Michigan. Diadromus subtilicornis (Gravenhorst) was identified parasitizing approximately 2% of DBM larvae (unpublished data). Percent of parasitism by D. insulare on DBM was 78.9%, 60.8% and 75.5% for the Eaton County greenhouse and the Monroe County field and the MSU Entomology Research farm, respectively (Table 2). The high percent of parasitism suggests that this parasitoid was abundant whenever its host exists in Michigan. The presence of low levels of parasitism of D. subtilicornis tends to agree with that reported by Harcourt (1960).

The sex ratio (male: female) of D. insulare adults was 1:1.7, 1:2.5 and 1:1.2 for Eaton County, Monroe County and the MSU Entomology farm, respectively (Table 2). Hologen (=Diadegma) insulare (Cresson) (Hymenoptera: Ichneumonidae), the principle parasitoid of DBM in eastern Ontario, Canada was reported to have sex ratios of 1.1:1.0 and 10:1.0 for field and laboratory reared adults, respectively (Harcourt 1960). However, the sex ratio for D. eucrophaga from field collected pupae was 1:3.1 (Delucchi et al. 1954). The sex ratio tended to be higher for the adult parasitoid emerging from pupae formed from surviving insecticide treated parasitized DBM larvae (Chapter 3).

The above-mentioned information suggests that the sex ratio is higher in the field than in the laboratory and varies from one location to another, probably influenced by the availability of food and/or weather.

High populations of D. insulare in and around fields as shown in this study and by Harcourt (1986), Bolter & Laing (1983), Lasota & Kok (1986) and Teetes & Randolph (1969), together with high parasitism rates on the host and

Table 2. Percent parasitism by Diadegma insulare (Cresson) on the diamondback moth, Plutella xylostella (L.) collected from three locations in Michigan.

Locations	Field collection (numbers) ¹			numbers of Pupae (reared from larvae collected)		% Parasitism	<u>D. insulare</u> Male:Female sex ratio
	DBM-L	DBM-P	DI-P	DBM-P	DI-P		
Eaton County	52	6	18	10	42	78.9	1:1.73
Monroe County	15	3	5	6	9	60.8	1:2.5
MSU Ent. Expt. field	203	8	50	57	144	75.5	1:1.2

1, DBM-L diamondback moth larvae, DBM-P = DBM pupae, DI-P = D. insulare pupae.

a high female sex ratio, should be manipulated and integrated with other tactics that would not seriously affect the parasitoid, but would bring down DBM populations below action threshold levels.

Field plot experiments. The mean numbers of DBM larvae per plant in each treatment before treatment were not significantly different ($P < 0.05$, Tukey's test, $F = 1.37$, $df = 13, 39$) but were significantly lower in all treated plots than in the untreated plots 3 d after insecticide spraying ($P < 0.05$, Tukey's test, $F = 14.25$, $df = 13, 39$) (Table 3).

There was no difference in the numbers of DBM pupae per plant among the treated plots before insecticide treatment (except between Javelin,

Table 3. Mean number of diamondback moth (DBM) larvae and D. insulare pupae per plant before (day 0) and after (day 3 or day 6) insecticide treatment.

Treatments	numbers of DBM larvae per plant ($\pm$ SE)		numbers of <u>D. insulare</u> pupae per plant ($\pm$ SE)	
	day 0	day 3	day 0	day 6
Javelin (1.8 kg/ha)	0.80 $\pm$ 0.08 a	0.34 $\pm$ 0.03 b	0.22 $\pm$ 0.02 a	0.12 $\pm$ 0.04 b
Javelin (1.4 kg/ha)	0.81 $\pm$ 0.03 a	0.46 $\pm$ 0.07 b	0.24 $\pm$ 0.05 a	0.30 $\pm$ 0.08 a b
CGA (3.7 kg/ha)	0.79 $\pm$ 0.01 a	0.32 $\pm$ 0.02 b	0.30 $\pm$ 0.04 a	0.13 $\pm$ 0.04 a b
CGA (7.4 kg/ha)	0.85 $\pm$ 0.02 a	0.33 $\pm$ 0.04 b	0.28 $\pm$ 0.01 a	0.19 $\pm$ 0.03 a b
MVP + Sticker (7.6 + 1.1) kg/ha	0.66 $\pm$ 0.02 a	0.28 $\pm$ 0.03 b	0.22 $\pm$ 0.05 a	0.20 $\pm$ 0.02 a b
MVP (7.6 kg/ha)	0.82 $\pm$ 0.03 a	0.32 $\pm$ 0.06 b	0.19 $\pm$ 0.03 a	0.26 $\pm$ 0.02 a b
MVP + Sunspray ultrafine oil (7.6 kg + 2.81)/ha	0.76 $\pm$ 0.07 a	0.27 $\pm$ 0.04 b	0.23 $\pm$ 0.12 a	0.26 $\pm$ 0.02 a b
permethrin (0.11 kg AI/ha)	0.68 $\pm$ 0.08 a	0.31 $\pm$ 0.03 b	0.24 $\pm$ 0.04 a	0.26 $\pm$ 0.05 a b
ICIA (0.02 kg AI/ha)	0.72 $\pm$ 0.03 a	0.27 $\pm$ 0.07 b	0.23 $\pm$ 0.02 a	0.18 $\pm$ 0.05 a b
esfenvalerate (0.03 kg AI/ha)	0.68 $\pm$ 0.03 a	0.03 $\pm$ 0.02 b	0.16 $\pm$ 0.06 a	0.13 $\pm$ 0.05 a b
thiodicarb (0.67 kg AI/ha)	0.73 $\pm$ 0.03 a	0.42 $\pm$ 0.09 b	0.29 $\pm$ 0.02 a	0.19 $\pm$ 0.03 a b
thiodicarb + Javelin (0.67 kg AI+1.8 kg)/ha	0.75 $\pm$ 0.05 a	0.29 $\pm$ 0.02 b	0.26 $\pm$ 0.04 a	0.28 $\pm$ 0.07 a b
chlorpyrifos (1.12 kg AI/ha)	0.74 $\pm$ 0.09 a	0.34 $\pm$ 0.06 b	0.33 $\pm$ 0.03 a	0.15 $\pm$ 0.03 a b
untreated	0.82 $\pm$ 0.04 a	0.79 $\pm$ 0.04 a	0.25 $\pm$ 0.04 a	0.36 $\pm$ 0.07 a

Values with the same letter in column are not significantly different ($P \leq 0.05$ by Tukey's test).

1.4 kg/ha plots with ICIA or chlorpyrifos) and 6 d after insecticide treatment ($P < 0.05$, Tukey's, $F = 3.04$, $df = 13, 39$). Similar results also showed between each treated plot with the untreated plot.

The change in number of DBM larvae per plant between each treated plot (0 vs 3 d after insecticide spraying) was not different but the change was significantly higher between each treated plot than the untreated plots (-0.025 ± 0.004) ($P < 0.05$, Tukey's test, $F = 5.04$, $df = 13, 39$). However, there was no difference in the change of DBM pupae, both among treated and between treated and untreated plots (0.084 ± 0.04) ($P < 0.05$, Tukey's test, $F = 3.54$, $df = 13, 39$).

None of the insecticides used in this study caused 100% larval mortality 3 d after treatment (Figure 1). Percent DBM larval mortality was not different among the treatments but was significantly higher in treated plots than untreated plots ($P < 0.05$, Tukey's test, $F = 11.02$, $df = 13, 39$). Factors like rainfall (Wakisaka et al. 1990, Harcourt 1986, Chelliah & Srivinasan 1986), entomophagous fungi (Srivapragasan et al. 1987, Wilding 1986, Tomiyama & Aoki 1982), and strong wind at night may greatly influence results. Rainfall can wash off small larvae or pesticide deposits and encourage the entomophagous fungi to attack both DBM larvae and pupae. Also, it may affect the role of oil or sticker added to the Bt and some differences in effectiveness may be expected between the three Bt products (MVP, Javelin and CGA) (Soares & Quick 1990).

The higher number of late instars in treated vs. untreated plots indicated that only early instars were much affected by insecticide spraying. The broccoli plants' canopy in each treatment may also have protected or provided a hiding place for some DBM larvae (Hoy et al. 1990), or caused less pesticide droplets to reach the underside of the leaves (Adams et al. 1990) or the leaves on the lower canopy. In Hawaii, Tabashnik (1986) estimated that

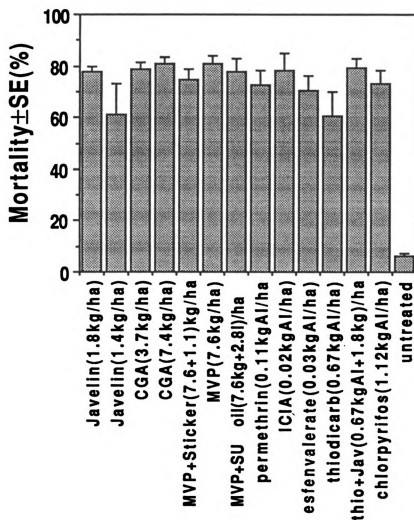


Figure 1. Mean percent mortality of diamondback moth larvae three days after insecticide treatment.

the percentage of larvae escaping insecticide exposure is between 0-20% of the total larval population. In the laboratory, some resistant larvae were also observed avoiding eating treated leaves and pupated early (Chapter 1). DBM larval mortality in the field studies seems to be a little bit lower than in the laboratory. This difference may be influenced by variable factors explained earlier.

To estimate the effect of insecticide treatments on D. insulare, the change in number of DBM and D. insulare pupae was calculated. The mean number of D. insulare pupae per plant was not significantly different between plots before insecticide treatment ($P < 0.05$, Tukey's test, $F = 0.85$, $df = 13,39$) (Table 3). Six days after insecticide spraying the numbers of D. insulare pupae in the untreated plots were significantly higher than in the plots treated with Javelin (1.8 kg/ha) ($P < 0.05$, Tukey's, $F = 2.34$, $df = 13,39$) indicating that some parasitized DBM larvae may be killed by the Javelin, causing less D. insulare to be formed. D. insulare pupae numbered less after treatment in nine of the twelve treatments but higher in the untreated plots, but variability was high and differences were not statistically significant. In addition to the environmental factors as discussed above, D. insulare adults are highly mobile and may distribute themselves throughout the field without being affected by individual plot treatments.

Results indicate that in this situation insecticides did not seriously affect the parasitism activity of D. insulare on DBM larvae. Parasitism after treatment ranged from 40.5-85.9% and only the two extremes were significantly different ($P < 0.05$, Tukey's test, $F = 1.80$, $df = 13,39$). The high variability and lack of treatment effect was probably due to movement of D. insulare adults among plots and border rows. This suggests that alternate row spraying (Casagrande & Haynes 1976) may functionally increase D. insulare

effectiveness in the field. Similar results were also reported in studies of DBM parasitism (Iman et al. 1986, Niwa et al. 1987). Mobility and distribution patterns of A. plutellae in the field were also reported unchanged after insecticide treatment (Sivapragesan et al. 1987). However, an unusually high rate of parasitism by Microplitis croceipes (Cresson) on bullworm larvae in fields treated with pyrethroids rather than organophosphates was reported by Powell et al. (1986). Grossman (1990) suggested that the behavioral response of the parasitized larvae toward pesticides applied could be a factor keeping the parasitism rate high. This somewhat supports our results because more than 50% of the DBM larvae population were late instars (more resistant or tolerant due to behavioral response toward the pesticides) which may mean most of them were parasitized by D. insulare (Harcourt 1986, Bolter & Laing 1983). Also, parasitized larvae consume leaves at a slower rate than healthy DBM, allowing residues to disintegrate through time and reduce mortality of the parasitized larvae (Chapter 3). The presence of vegetation that provides refuges for D. insulare adults is probably the biggest factor causing the variability in parasitism rate as reported by Sastrosiswojo & Sastrodiharjo (1986) and Lim et al. (1986), on the parasitism of D. eucero-phaga and A. plutellae, respectively.

Bt is not toxic to D. insulare adults but may indirectly kill the immature stages of D. insulare (Chapters 1 and 3). The high numbers of DBM larvae killed by Bt (Chapter 1) could reduce the number of hosts to be parasitized by D. insulare. This will indirectly reduce the future populations of D. insulare. Intense selection by Bt may produce much higher levels of resistance due to intrapopulation genetic variation in susceptibility to Bt (Tabashnik et al. 1991). Also, Bt is very expensive in most third world countries (Salinas 1986, Ooi 1986). To overcome these problems, we should

let D. insulare control DBM first and use Bt when DBM larval populations are above the action threshold level (ATL) (Sastrosiswojo & Sastrodiharjo 1986). In fact, the ATL could be increased because parasitized DBM larvae cause less damage than non-parasitized larvae (Chapter 3, Parker 1974). The use of carbamate like thiodicarb which is as effective as Bt or pyrethroid insecticides (Figure 1) (Croft & Whalon 1982, Powell & Scott 1985) would favor parasitoids. DBM adults easily escape from insecticide spraying and it acts as a source for future resistance (Moore & Tabashnik 1989). Bt could not control DBM adults. Therefore, a mixture of Bt-thiodicarb or esfenvalerate or cypermethrin could control both DBM larvae and adults with minimal impact on D. insulare adults (Iman et al. 1986). The spraying should be carried out late in the evening (Chelliah & Srivinasan 1986) where DBM adults are active but there is less parasitoid activity (Powell et al. 1984).

Percent of parasitism in guard rows. The change in parasitism in the guard rows 0 vs. 6 d after treated rows were sprayed with insecticides was not significant ($P < 0.05$, t-test) (Table 4). This again indicates that parasitism is apparently not seriously affected by insecticide spraying in an alternate row situation.

Insecticide spraying in this study probably killed some D. insulare adults (Chapter 1). In part, this may be compensated for by less mutual interference between searching females (Bolter & Laing 1983, Putnam 1986, Rogers 1972) and higher parasitism per female. Lower numbers of DBM larvae in the field might also increase the percent of parasitism by D. insulare (Bolter & Laing 1983) providing there are refuges in or around the field. Sunflowers planted at edges of fields were found to be the key element in integrated pest control in approximately 4,060 ha of heavily insecticide treated processing tomato fields in the Dominican Republic (Grossman 1990).

Table 4. Change in the number of diamondback moth (DBM) and D. insulare pupae and percent parasitism per plant in 10 guard rows at 0 vs 6 days after spraying of insecticides on the treated rows.

	Change in number of DBM pupae	Change in number of <u>D. insulare</u> pupae	Change in % parasitism of DBM by <u>D.</u> <u>insulare</u>
Mean at 0 vs. 6 d	0.42 vs. 0.63	1.05 vs. 1.79	70.20 vs. 73.10
mean change	0.249	0.752	3.08
SE	0.091	0.339	1.77
t	2.73	2.218	1.74
P	< 0.05 (t-test)	ns	ns

Yellow rocket, Barbarea vulgaris R.Br. (Harcourt 1986) and black mustard, Brassica nigra L. (Kock) found in the surrounding field served as a wild host for DBM and D. insulare. Also, high volume nectar and pollen producing plants planted in adjacent research plots by Dr. George Ayers provided a food source and refuge for D. insulare adults. The highest number of parasitoid species and percent of parasitism of H. zea larvae by M. croceipes occurs in early season crops, indicating that existence of wild plants which provide food for the pest (indirectly providing hosts for the parasitoid) are not seriously affected by pesticides which are not yet heavily applied in early season crops (Stadelbacher et al. 1984).

This small plot may somewhat simulate the situation of a small cabbage field or garden where the surrounding area normally includes other wild plants or crops that serve as refuges for the parasitoids.

Field cage experiment. There were no significant differences between treatments in the numbers of DBM and D. insulare pupae formed from larvae collected before insecticide application ($P < 0.05$, Tukey's test; DBM, $F = 0.24$ & D. insulare, $F = 246.0$; $df = 3, 6$) (Table 5).

After insecticide treatment, the numbers of D. insulare pupae in the control and Bt treated cages were significantly higher than in permethrin or azinphosmethyl treated cages ($P < 0.05$, Tukey's test, $F = 226.25$, $df = 3, 6$). The reverse result was obtained for DBM pupae ($P < 0.05$, Tukey's test, $F = 0.35$, $df = 3, 6$). Permethrin and azinphosmethyl apparently killed D. insulare adults but not DBM larvae (released 4 d after spraying). Therefore, fewer DBM larvae were parasitized and more DBM pupae formed. In laboratory bioassays, permethrin and azinphosmethyl caused 100% mortality to D. insulare adults even at a very low concentration (Chapter 1). However, parasitism still occurred in cages treated with these two insecticides (Table 5). Some D. insulare escaped or survived the impact of permethrin and azinphosmethyl. The most probable explanation is that some existing D. insulare pupae were not entirely collected before the experiment started (see Materials & Methods). The pupae of D. insulare are less sensitive than the larvae to these two insecticides (Chapter 3) and D. insulare lay eggs as soon as 24 h after emerging as adults (Bolter & Laing 1983). Apanteles marginiventris (Cresson) (Hymenoptera: Braconidae) (Lingren et al. 1972) and A. plutellae (Lim et al. 1986) pupae are also less sensitive to insecticide treatment than adults. However, the escaped D. insulare would probably not survive if

Table 5. Mean number of diamondback moth (DBM) and Diadegma insulare (Cresson) pupae and percent of parasitism by D. insulare on DBM per plant in field cage experiments.

Treatments	Before spray			After spray		
	Number of pupae/plant		% parasitism ($\pm$ SE)	Number of pupae/plant		% parasitism ($\pm$ SE)
	DBM ($\pm$ SE)	<u>D. insulare</u> ($\pm$ SE)		DBM ($\pm$ SE)	<u>D. insulare</u> ($\pm$ SE)	
permethrin	14.3 (2.0) a	45.7 (1.9) a	76.1 (4.3) a	55.3 (2.4) a	4.7 (2.4) b	7.8 (3.6) b
<u>B. thuringiensis</u>	13.0 (2.1) a	47.0 (2.1) a	78.3 (3.8) a	11.0 (1.1) b	48.3 (1.3) a	81.5 (3.3) a
azinphosmethyl	15.7 (2.2) a	43.7 (2.4) a	73.6 (5.6) a	52.0 (1.5) a	8.0 (1.5) b	13.3 (2.3) b
untreated	14.6 (1.7) a	45.3 (1.8) a	75.6 (4.2) a	12.3 (1.2) b	47.3 (0.9) a	79.4 (2.2) a

Means with same letter in column are not significantly different ($P < 0.05$, Tukey's test).

insecticides were applied again a few days later as is generally practiced by cabbage growers.

Results of this experiment would somewhat simulate a larger field situation where the parasitoids are in the field most of the time and there are few refuges for them to hide and fewer outside sources of pollen and nectar. In the field, therefore, the application of insecticides should not be too frequent so that some D. insulare pupae have time to emerge and attack new hosts.

Conclusion

Results of my field studies indicated that all insecticides were equally effective against DBM larvae and had no apparent adverse effect on the survival and parasitism activity of D. insulare. However, insecticide spraying was done only once and alternate rows were left unsprayed. Cabbage growers usually apply insecticides every 5-10 days during the cropping season which could eliminate D. insulare from cabbage fields within a short time as was shown in the field cage studies.

The need for insecticides in DBM control, however, is still accepted worldwide (Lim et al. 1986). The ability to kill DBM at high numbers within a short time is the primary reason that insecticides still play a major role in managing DBM in the field. However, the ability of DBM to develop resistance to any newly introduced insecticide, especially to the more toxic ones (Cheng 1988), has caused a serious problem to cabbage growers (Lim et al. 1986).

A good understanding about field ecosystems, the condition of the surrounding field, level of susceptibility and the behavior of DBM larval and

adult populations, the selectivity or toxicity of insecticides to both DBM larvae and adults as well as D. insulare could ensure the presence of D. insulare in cabbage agroecosystems and provide good DBM control with less cost. The use of Bt as well as a mixture of Bt with other insecticides less toxic toward D. insulare adults, but effective against the DBM adults and/or larvae, might be one of the best combinations that could be incorporated with other tactics for successful DBM management in cabbage fields. Also, the size of the field and the management of other DBM hosts and D. insulare hosts and adult food sources outside the field will affect overall DBM abundance and insecticide resistance and the effectiveness of D. insulare. In addition to effects at the individual farm level, using D. insulare in integrated DBM management will slow down resistance development in DBM on a regional scale.

Chapter 3

Indirect effects of insecticides on the immature stages
of Diadegma insulare (Cresson) (Hymenoptera: Ichneumonidae),
a parasitoid of the diamondback moth,
Plutella xylostella (L.) (Lepidoptera: Plutellidae)

ABSTRACT: The indirect effect of pesticides on the immature stages of Diadegma insulare (Cresson), a parasitoid of the diamondback moth (DBM), Plutella xylostella (L.) was studied in the laboratory. DBM larvae parasitized by D. insulare were significantly less sensitive to ingested pesticides than were non-parasitized larvae 48 h after treatment. However, they were equally sensitive to pesticides through contact. Bacillus thuringiensis (Bt) and azinphosmethyl were significantly more toxic to parasitized larvae than permethrin, methomyl and chlorothalonil in a leaf dip bioassay. Methomyl was more toxic to parasitized larvae than non-parasitized larvae. Mortality of parasitized larvae was lower than non-parasitized larvae at the same concentration 24 h but approximately similar 48 and 72 h after treatment. The sex ratio of D. insulare adults emerging from the pupae that formed from the surviving insecticide-treated larvae tended to have more female adults. D. insulare adult emergence from the insecticide (except with permethrin at 10.0 mg AI/ml concentration) treated pupae were not severely affected. Bt and chlorothalonil had no effect on DBM and D. insulare emergence. The less sensitive immature stages of D. insulare toward pesticides should be manipulated for effective integrated DBM management.

Key words: Insecta, D. insulare, diamondback moth, toxicity, parasitized.

The diamondback moth (DBM), Plutella xylostella (L.), is a serious insect pest of crucifers and one of the most resistant insect species to insecticides, including resistance to organochlorines, organophosphates, carbamates, pyrethroids, insect growth regulators and lately to Bacillus thuringiensis (Shelton & Wyman 1990, Tabashnik et al. 1990a, 1990b & 1991, Perng et al. 1988, Sun et al. 1983, Barroga 1980, Magallona 1977-78). Excessive use of insecticides often results in pest resurgence, elevating other potential pests to major pest status, increasing environment pollution and destroying the biological control agents that regulate the population of DBM in crop agro-ecosystems (Lim et al. 1986, Lim 1986).

Several biological control agents, especially the parasitoids, Diadegma insulare (Cresson) (Hymenoptera: Ichneumonidae) (Harcourt 1969, 1986, Lasota & Kok 1986, Putnam 1968, Bolter & Laing 1983), Diadegma eucrophaga (Horstmann) (Hymenoptera: Ichneumonidae) (Sastrosiswojo & Sastodiharjo 1986, Iman et al. 1986, Ooi 1986), Diadegma semiclausum (Hellen) (Hymenoptera: Ichneumonidae) (Poelking 1990), Diadegma plutellae (Viereck) (Hymenoptera: Ichneumonidae) (Parker 1974), Apanteles plutellae Kurdjumov (Hymenoptera: Braconidae) (Lim & Ko 1975, Ooi 1986), Microplitis plutellae (Muesbeck) (Hymenoptera: Braconidae) (Putnam 1968), and Diadromus subtilicornis (Gravenhorst) (Hymenoptera: Ichneumonidae) (Harcourt 1986) have been reported to play an important role in bringing down the DBM larval population in the cabbage fields. In southern Ontario, Canada, D. insulare parasitizes up to 75% of DBM larvae (Harcourt 1969). In Indonesia, D. eucrophaga is an important biological control agent for DBM (Sastrodiharjo 1986, Iman et al. 1986). DBM parasitoids were also reported to have caused the DBM larval population left between 1-5 per 20 cabbage plants (Parker 1974). The high rate of host mortality achieved by D. insulare and

other DBM parasitoids indicates that there is the potential of using them as one of the components in integrated DBM management.

Insecticides continue to be the major means for controlling pests (Lingren et al. 1972). Most insecticides have detrimental impacts on biological control agents. However, some of the insecticides used in cotton production have been shown to possess either differential or selective toxicity to certain beneficial insects (Lingren & Ridway 1967, Cate et al. 1972, Powel et al. 1986). In cabbage agro-ecosystems, D. eucero-phaga (Iman et al. 1986, Sastrosiswojo & Sastodiharjo 1986, Santoso 1979) and Apanteles plutellae (Kurdjumov) (Lim et al. 1986, Ooi 1986) have been reported to adapt to an environment of frequent pesticide application and their rate of parasitism on DBM was not adversely affected. D. insulare's presence and role in the cabbage agro-ecosystem was described in detail by Harcourt (1986), but its response to insecticides has only recently been studied (Chapters 1 and 2). Laboratory studies on D. eucero-phaga (Iman et al. 1986) and on D. insulare (Chapter 1) indicate that all insecticides tested except Bt were extremely toxic to parasitoid adults. However, parasitism of D. eucero-phaga was not adversely affected by insecticides in two of the three insecticide-treated fields (Sastrosiswojo & Sastrodiharjo 1986). Similar results were also reported for parasitism of D. semiclausum and A. plutellae on DBM (Poelking 1990, Ooi 1986, Lim et al. 1986).

In addition to direct effects on parasitoid adults, insecticides may indirectly affect parasitoid larvae within their host. For instance, Apanteles melanoscelus Ratzeburg (Hymenoptera: Braconidae) larvae within its host larvae (gypsy moth) are indirectly killed by the carbaryl and Bacillus thuringiensis (Ahmad et al. 1978, Ahmad & Forgash 1976, Thorpe et al. 1990). However, gypsy moth larvae parasitized by A. melanoscelus are less sensitive

to these two insecticides than non-parasitized larvae, indicating that the parasitoid larvae may be protected by the host from the impact of pesticides.

The impact of insecticides on the immature stages of the genus Diadegma has not been investigated. Larvae of D. insulare within the parasitized DBM larvae may be less sensitive to insecticides than adult D. insulare. Pupation of D. insulare occurs within the DBM pupa and again may be somewhat protected from contact with insecticides, as was reported by Chelliah & Srinivasan (1986) for A. plutellae pupae in DBM pupae. D. insulare larvae may also be indirectly affected by Bt through ingestion by the DBM larvae even though Bt does not affect adult D. insulare.

The objective of this study was to investigate the response of immature stages of D. insulare to insecticides that are commonly used for DBM control in cabbage fields. Information will be important for integrating D. insulare into management of DBM.

Materials and Methods

DBM rearing. For the source of DBM and resistance status refer to Chapter 1. A 200 ml plastic cup nearly filled with water was used to hold young broccoli leaves. Holes were made in the cup cover and stems of 8-9 young broccoli leaves were inserted into the cup through the hole. The cup and the leaves were placed inside a 45 x 35 x 35 cm ovipositional cage (21±2°C, 16:8 L:D photoperiod). DBM pupae (reared as in Chapter 1) were then placed inside the cage for adult emergence. A muslin cloth wetted with diluted honey was put in a small petri dish inside the cage to feed the DBM adults. A few drops of diluted honey were added to the muslin cloth every 2 days. After 2 days, the leaves with eggs were transferred to a 30 x 20 x 15 cm deep

cake pan, with a 6 x 6 cm screen in the lid, and kept at $21 \pm 2^{\circ}\text{C}$ to hatch and for rearing. The larvae were fed fresh broccoli leaves every two days. These larvae were used for bioassays, D. insulare parasitism (to get the parasitized DBM larvae), or reared until pupation. Pupae of DBM were collected and kept at 5°C for further rearing.

D. insulare rearing. Stems of four mature broccoli leaves were inserted into the 200 ml plastic cup through the hole (prepared as above). The leaves were inoculated with 2nd or 3rd instar DBM (from above). The plastic cups with the inoculated leaves were placed in rearing cages as before. D. insulare pupae (ca. 100 per cage) were placed inside the cage for adult emergence. D. insulare adults were fed diluted honey, as before. Leaves with 3rd to 4th instar DBM were collected about two days later and the (parasitized) larvae were transferred to a modified cake pan for experimental use or rearing as above until pupation, for future rearing.

Pesticides used. Commercial formulations of permethrin (Ambush 2 EC, ICI Americas, Inc., Wilmington DE), azinphosmethyl (Guthion 3F, Mobay Corp., Kansas City MO), methomyl (Lannate 1.8L, DuPont de Nemours, Wilmington DE), Bacillus thuringiensis Berliner var. kurstaki (Dipel 2X, Abbott Laboratories, North Chicago IL) and the fungicide, chlorothalonil (Bravo 720, Fermenta ASC Corp., Mentor OH) were used. With the exception of guthion, they represent the most commonly recommended pesticides in Michigan for control of insect pests and diseases of cabbage, broccoli and other cruciferous crops (Guthion is no longer registered for use on crucifers).

Bioassays

Larvae. Leaf dip bioassays were carried out to determine the response of parasitized and non-parasitized DBM larvae to ingestion of the pesticides listed above. Leaves (small enough to fit a 14.5 cm diameter plastic petri dish) were cut from frame leaves of uninfested broccoli raised in the greenhouse. Individual leaves were randomly selected, immersed in the pesticide solution (100, 10, 1.0, 0.1 or 0.01 mg active ingredient (AI)/ml of water) for three seconds and air dried for 2 h. Control leaves were treated with tap water. The treated leaves were placed on top of a filter paper (one per dish) in a 14.5 cm diameter petri dish and five randomly-selected presumably parasitized 3rd instar DBM were released in each petri dish and held at $21 \pm 2^{\circ}\text{C}$ and 16:8 (L:D) photoperiod. The treatments were replicated four times (four petri dishes for each concentration) and larval mortality was recorded at 24, 48 and 72 h. Larvae were considered dead if they could not walk one body length when gently probed. Similar treatments were done on non-parasitized 3rd instar DBM (in separate dishes).

Mortality of both parasitized (presumably parasitized) and non-parasitized DBM larvae on treated leaves was corrected for mortality on treated leaves (with non-parasitized larvae) (Thorpe et al. 1990) using Abbott's (1925) formula. Number of larvae surviving, pupae formed, adults emerged from pupae and sex ratio were observed. Data were analyzed using probit analysis and analysis of variance (MSTAT, Eisensmith & Russel 1989). LC_{50} values were statistically compared using overlap or non-overlap of 95% confidence limits. However, the sex ratio of D. insulare was statistically compared using the chi-square test.

Direct dipping of larvae into the pesticide solution was used to study the difference in sensitivity of parasitized and non-parasitized DBM larvae to insecticides through contact. Five randomly-selected parasitized or non-parasitized 3rd instar DBM were placed in a 4 cm diameter tea strainer and dipped in prepared pesticide solutions (100, 10, 1.0, 0.1 or 0.01 mg AI/ml of water) for three seconds. Tap water was used as control. Larvae were taken out of the tea strainer, blotted dry on paper towels and released on untreated broccoli leaves in 14.5 cm diameter petri dishes as before. Concentrations were replicated four times each for parasitized and non-parasitized larvae. Mortality, number of larvae surviving, pupae formed, D. insulare emerged and sex ratios for parasitized DBM larvae and mortality for non-parasitized larvae were recorded. Data were analyzed as before.

Pupae. Direct dipping of DBM and D. insulare pupae into the pesticide solution was used to study the effect of each pesticide on adult emergence from treated pupae and mortality of emerged adults 24 h after emergence. Ten randomly-selected 3 to 4 day old D. insulare pupae or DBM pupae were placed inside the tea strainer and dipped in a prepared pesticide solution (10.0, 1.0 or 0.1 mg AI/ml) for three seconds. Tap water was used for control. Pupae were taken out of the tea strainer, blotted dry on paper towels and placed in 18 cm x 15 mm test tubes. The tops of the test tubes were closed with cotton balls and diluted honey was supplied as food for the emerged adults through the cotton plug. The pupae were held in a growth chamber at $25 \pm 2^{\circ}\text{C}$, 55% R.H and 16:8 L:D photoperiod. The pesticide concentrations were replicated four times (four test tubes, ten pupae per test tube).

To determine if a foliar spray might show results similar to the direct dip bioassay, a hand held CO₂ sprayer was used to study the toxicity of permethrin, azinphosmethyl and methomyl on the DBM and D. insulare

pupae. Ten randomly-selected 3 to 4 day old D. insulare pupae were placed on paper towels and sprayed with a hand held CO₂ sprayer containing a pesticide solution (1.0 mg AI/ml concentration) at 6.4 ml/sec, 458 l/ha with a flat fan TeeJet nozzle (0.3 m distance between the nozzle and the pupae) for 2 to 3 seconds. The treated pupae were then transferred into the test tubes and held as before. Percent of adults emerged was recorded until at least 95% emergence occurred in the control. Mortality of the emerged adults was also observed 24 h after emergence. Data were analyzed by analysis of variance (MSTAT, Eisensmith & Russell 1989).

Time for pupation and adult emergence. This study was designed to measure any difference in time required for parasitized and non-parasitized DBM larvae to pupate and D. insulare and DBM adults to emerge. Ten parasitized and ten non-parasitized 3rd instar DBM (four replications of each) were randomly selected and released in 14.5 cm diameter petri dishes with fresh broccoli leaves and held at $21 \pm 2^{\circ}\text{C}$. Larvae were fed fresh broccoli leaves every two days. Days for pupation and adult emergence from pupae were recorded. Data was analyzed using Student t-test (MSTAT, Eisensmith & Russell 1989).

Results and Discussion

Larvae. Mortality of parasitized DBM larvae in the leaf dip bioassay when treated with 1.0 mg AI/ml of methomyl or permethrin was significantly lower than for non-parasitized larvae ($P < 0.05$, t-test, $df=6$) (Figure 1 A & B). The active movement of the non-parasitized larvae on the leaf treated with methomyl (1.0 & 10.0 mg AI/ml concentrations) and permethrin rather than the leaf treated with azinphosmethyl and Bt may be the factor that

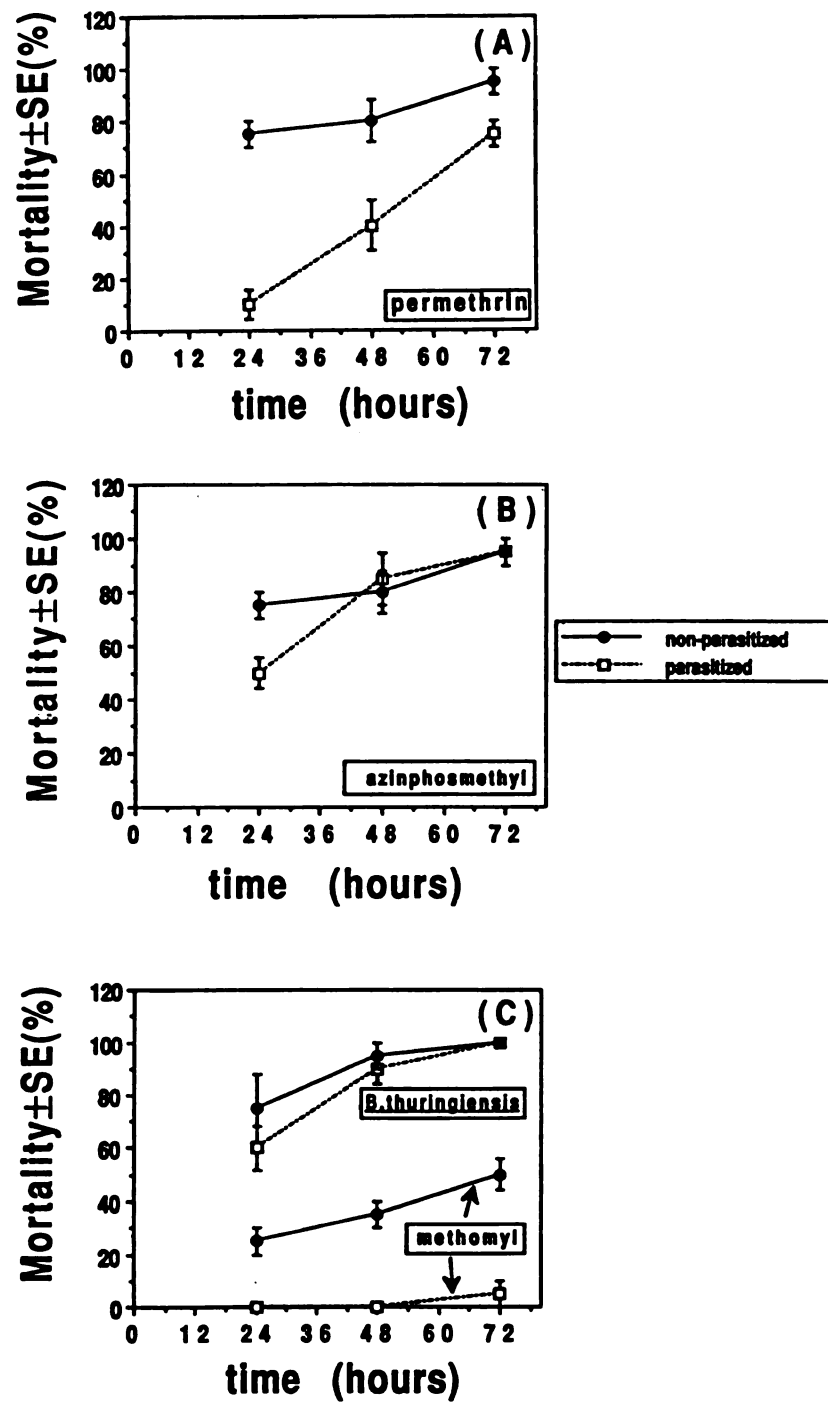


Figure 1. Mortality of non-parasitized larvae diamondback moth and larvae parasitized by *D. insulare* after being exposed to 1.0 mg AI/ml insecticide solution at 24, 48 and 72 hours after treatment in leaf dip bioassay.

caused higher mortality of the non-parasitized larvae (Figure 1A, B & C). However, mortality of parasitized and non-parasitized DBM larvae seems to be similar at 48 and 72 h for Bt and azinphosmethyl and 72 h for permethrin ($P < 0.05$, t-test, $df = 6$). The joint action of both D. insulare larvae that weaken the host larvae and the accumulative effect of toxic residues of these pesticides consumed by the host larvae could be the best explanation as reported by Ahmad et al. (1978) on the gypsy moth larva parasitized by A. melanoscelus treated with Bt. This action may not be working well in the field because the parasitized larvae will consume pesticide residues on the leaf that disintegrate through time. The fungicide chlorothalonil did not cause mortality to parasitized or non-parasitized DBM larvae at 1.0 mg AI/ml 72 h after treatment, and at 100 mg AI/ml concentration caused 15% and 10% mortality to parasitized and non-parasitized DBM larvae, respectively.

The difference of LC_{50} values between parasitized and non-parasitized DBM larvae (Table 1) also indicated that all of the insecticides affect parasitized and non-parasitized larvae differentially. Parasitized larvae were observed to feed less than non-parasitized larvae in the permethrin and methomyl treatments, probably accounting for the differences. Parasitized larvae treated with azinphosmethyl had significantly higher LC_{50} values than did the non-parasitized larvae at 48 and 72 h after treatment. Again, this might be due to the joint action of D. insulare larvae within the host and the accumulative effect of pesticides consumed by the host larvae. Although Bt is also toxic if ingested, there was no significant difference in LC_{50} values on parasitized and non-parasitized larvae. This probably was due to Bt having no contact action as possessed by azinphosmethyl. The slight contact toxicity of azinphosmethyl will further weaken the parasitized larvae. However, Bt seems to be the most toxic of the pesticides tested to both parasitized and

Table 1. Response of non-parasitized and *D. insulare* parasitized diamondback moth larvae to insecticides at 24, 48 and 72 h after treatment in leaf dip bioassay.

Pesticides ^a		Time (hours)		
		24	48	72
permethrin parasitized	LC ₅₀ (n=100)	3.99	1.384	0.400
	95% C.L.	3.832 - 4.188	1.288 - 1.488	0.368 - 0.436
	Slope $\pm$ SE	2.106 $\pm$ 0.095	1.263 $\pm$ 0.021	1.091 $\pm$ 0.014
	Chi-square (df=3)	0.036	0.532	1.505
non-parasitized	LC ₅₀ (n=100)	0.112	0.074	0.037
	95% C.L.	0.112 - 0.142	0.064 - 0.085	0.033 - 0.042
	Slope $\pm$ SE	0.902 $\pm$ 0.011	0.859 $\pm$ 0.012	0.989 $\pm$ 0.019
	Chi-square (df=3)	2.253	2.256	1.973
azinphosmethyl parasitized	LC ₅₀ (n=100)	1.00	0.112	0.029
	95% C.L.	0.925 - 1.081	0.102 - 0.123	0.024 - 0.034
	Slope $\pm$ SE	1.166 $\pm$ 0.016	1.075 $\pm$ 0.017	0.909 $\pm$ 0.018
	Chi-square (df=3)	0.690	1.805	5.162
non-parasitized	LC ₅₀ (n=100)	0.319	0.169	0.094
	95% C.L.	0.262 - 0.349	0.157 - 0.183	0.087 - 0.100
	Slope $\pm$ SE	1.027 $\pm$ 0.013	1.189 $\pm$ 0.019	1.318 $\pm$ 0.028
	Chi-square (df=3)	0.921	0.810	2.041
methomyl parasitized	LC ₅₀ (n=100)	30.890	18.961	4.929
	95% C.L.	28.352 - 33.655	17.713 - 20.295	4.605 - 5.277
	Slope $\pm$ SE	1.278 $\pm$ 0.036	1.415 $\pm$ 0.037	1.340 $\pm$ 0.026
	Chi-square (df=3)	1.509	2.011	5.579
non-parasitized	LC ₅₀ (n=100)	2.990	1.937	0.885
	95% C.L.	2.751 - 3.251	1.790 - 2.098	0.822 - 0.951
	Slope $\pm$ SE	1.100 $\pm$ 0.015	1.152 $\pm$ 0.016	1.249 $\pm$ 0.020
	Chi-square (df=3)	2.700	1.145	0.509
<i>B. thuringiensis</i> parasitized	LC ₅₀ (n=100)	0.501	0.011	0.004
	95% C.L.	0.445 - 0.565	0.009 - 0.013	0.002 - 0.005
	Slope $\pm$ SE	0.881 $\pm$ 0.011	1.116 $\pm$ 0.017	0.903 $\pm$ 0.015
	Chi-square (df=3)	0.634	0.947	1.082
non-parasitized	LC ₅₀ (n=100)	0.283	0.008	0.002
	95% C.L.	0.114 - 0.452	0.007 - 0.009	0.0015 - 0.0037
	Slope $\pm$ SE	0.759 $\pm$ 0.070	0.777 $\pm$ 0.010	0.861 $\pm$ 0.015
	Chi-square (df=3)	2.647	1.451	4.640

^a LC₅₀ for pesticides was mg AI/ml and at 100 mg AI/ml concentration, chlorothalonil only caused 15% & 10% mortality on diamondback larvae parasitized and non-parasitized by *D. insulare* 72 h after treatment.

non-parasitized larvae. As far as the non-parasitized larvae is concerned, these results tended to agree with that reported in Chapter 1.

The numbers of D. insulare pupae formed from the surviving parasitized DBM larvae treated with 1.0 mg AI/l pesticide solution in the leaf dip bioassay were significantly lower in Bt, permethrin and azinphosmethyl treatments than in methomyl, chlorothalonil treatments or in the control (Table 2). No larvae survived the Bt treatment. Again, this may be due to the joint action of D. insulare larvae within the host and the accumulative toxic effect of pesticides consumed by the host larvae. This is similar to the results of Culin & Dubose (1987) where chlordimeform, methyl parathion and esfenvalerate significantly reduced the percent of the Microplitis demolitor (Wilkinson) parasitized larvae surviving to parasitoid pupation, without affecting the non-parasitized host larvae, Heliothis zea (Boddie).

Except for the methomyl treatment, all parasitized larvae surviving from insecticide treatments successfully formed D. insulare pupae, indicating that the pesticides had no adverse impact on D. insulare larvae within the host body. In the methomyl treatment two of the emerged D. insulare could not fly well and appeared to be weaker than the others. Of 18 surviving larvae in the methomyl treatment, three formed DBM pupae rather than D. insulare pupae, indicating that methomyl had a slight direct toxicity effect on D. insulare larvae within the DBM host. No DBM pupae developed from untreated larvae or larvae surviving any other treatment.

Pesticides used in this study apparently altered the sex ratio of the D. insulare (Table 2). The sex ratios (male: female) of the D. insulare adults in permethrin, azinphosmethyl, methomyl and Bt treatments were significantly lower (more female) than in chlorothalonil or control treatments ($P < 0.05$, chi-square test, $df=3$). The carbamate and carbaryl also altered the sex ratio of

Table 2. Mean number of diamondback moth (DBM) larvae parasitized by D. insulare surviving 72 hours after treatment with a 1.0 mg AI/ml pesticide solution (leaf dip bioassay), pupae formed from the surviving larvae, adults emerged from the pupae and their sex ratio.

Insecticides	Numbers of DBM larvae surviving ($\pm$ SE) ^{1,2} (1)	Numbers of <u>D. insulare</u> pupae formed ($\pm$ SE), from (1) ¹ (2)	Numbers of <u>D. insulare</u> adults emerged ($\pm$ SE), from (2) ¹ (3)	Sex ratio (male: female) <u>D. insulare</u> from (3)
permethrin	0.75 $\pm$ 0.25 b	0.75 $\pm$ 0.25 b	0.75 $\pm$ 0.25 b	1.0: 1
azinphosmethyl	0.25 $\pm$ 0.25 b	0.25 $\pm$ 0.25 b	0.25 $\pm$ 0.25 b	0: 1
methomyl	4.75 $\pm$ 0.57 a	3.73 ³ $\pm$ 0.29 a	3.50 ⁴ $\pm$ 0.28 a	2.5: 1
<u>B. thuringiensis</u>	0 b	0 b	0 b	–
chlorothalonil	4.25 $\pm$ 0.57 a	4.25 $\pm$ 0.57 a	4.25 $\pm$ 0.57 a	4.3: 1
control	5.00 a	5.00 a	4.75 $\pm$ 0.57 a	3.7: 1

¹ Values in a column with same letter are not significantly different ($P < 0.05$, Tukey's test).

² Out of five larvae per replication (for instance, 0.75 $\times$ 4 replicates = 3 larvae survived/ treatment).

³ Three DBM pupae (non-parasitized) were also formed.

⁴ Two DBM adults also emerged out of original 19.

A. melonesculos, a parasitoid of the gypsy moth (Ahmad & Forgash 1976), and Rosenheim & Hoy (1988) reported that chlorpyrifos shifted the sex ratio of Aphytis melinus DeBach more toward females. The surviving parasitized larvae were probably healthier than the non-surviving larvae. The healthier larvae are good quality hosts which would determine the fecundity of D. insulare females. Therefore, insecticide treatment explained that host quality may play a part in the sex determination of D. insulare. Although the overall

mortality was higher in the permethrin and azinphosmethyl treatments, an increase in the number of females would be highly beneficial.

In the direct dip bioassay, LC₅₀ values for each pesticide (except methomyl) were not significantly different for parasitized and non-parasitized larvae (overlapping of 95% confidence limits) (Table 3), indicating that both parasitized and non-parasitized larvae had similar sensitivity to pesticides through contact. The LC₅₀ value for methomyl with parasitized larvae 48 h after treatment was significantly lower than non-parasitized larvae (Table 3). This was the exact opposite of the results in the leaf dip bioassay, that is, parasitized DBM larvae appeared to be more sensitive through contact with methomyl than non-parasitized larvae, but less sensitive through ingestion. In the direct dip bioassay, each larvae received exactly the same exposure (except for variations in size and surface area). In the leaf dip bioassay, parasitized larvae were observed to feed much less often than non-parasitized larvae, ingesting less pesticide.

Bt caused only 20% and 15% mortality even at the 100 mg AI/ml concentration (72 h) to parasitized and non-parasitized DBM larvae, respectively. This mortality was probably due to ingestion during the dipping treatment. Bt is only effective if ingested (Fast & Donaghue 1971, Sabesta et al. 1981).

The sex ratio (male: female) of the emerging D. insulare adults in the azinphosmethyl treatment was significantly different (no females) from the sex ratios in the other treatments ($P < 0.05$, chi-square, $df = 3$) (Table 4). It is unclear why no females emerged after azinphosmethyl treatment, however this could be very detrimental if such a differential effect occurred in the field.

Pupae. Percent D. insulare emergence from pupae treated with permethrin, methomyl or azinphosmethyl were significantly lower than

Table 3. Response of non-parasitized and D. insulare parasitized diamondback moth larvae to insecticides 48 hours after treatment in a direct dip bioassay.

Pesticides	LC ₅₀ (n) ^a	95% C.L.	Slope ± SE	Chi-square (df=3)
permethrin				
parasitized	0.002 (100)	0.001 - 0.007	0.710 ± 0.022	0.237 (3)
non-parasitized	0.004 (100)	0.002 - 0.009	0.669 ± 0.016	2.751 (3)
azinphosmethyl				
parasitized	0.003 (100)	0.002 - 0.009	0.654 ± 0.015	0.770 (3)
non-parasitized	0.002 (100)	0.001 - 0.012	0.649 ± 0.014	0.929 (3)
methomyl				
parasitized	3.980 (100)	3.415 - 4.432	0.810 ± 0.009	2.865 (3)
non-parasitized	10.069 (100)	8.197 - 12.369	0.651 ± 0.007	3.506 (3)
<u>B. thuringiensis</u>				
parasitized	>100 ^b (100)	-	-	-
non-parasitized	>100 ^c (100)	-	-	-
chlorothalonil				
parasitized	9.846 (100)	.777 - 11.058	.940 ± 0.016	3.581 (3)
non-parasitized	8.539 (100)	7.453 - 9.783	0.817 ± 0.090	4.377 (3)

a mg AI/ml.

b 72 h after treatment with 100 mg AI/ml, mortality was 25%.

c 72 h after treatment with 100 mg AI/ml, mortality was 20%.

Table 4. Mean number of diamondback moth (DBM) larvae parasitized by D. insulare surviving 72 hours after treatment with 1.0 mg AI/ml pesticide solution (direct dip bioassay), pupae formed from the surviving larvae, adults emerged from the pupae and their sex ratio.

Insecticides	Numbers of DBM larvae surviving (±SE) ^{1,2} (1)	Numbers of <u>D. insulare</u> pupae formed (±SE), from (1) ¹ (2)	Numbers of <u>D. insulare</u> adults emerged (±SE), from (2) ¹ (3)	Sex ratio (male: female) <u>D. insulare</u> from (3)
permethrin	0 b	0 b	0 b	-
azinphosmethyl	1.00 ± 0.25 b	1.0 ± 0.25 b	1.00 ± 0.25 b	1.0: 0
methomyl	3.75 ± 0.64 a	3.75 ± 0.64 a	3.50 ± 0.25 a	3.7: 1
<u>B. thuringiensis</u>	3.75 ± 0.28 a	3.50 ± 0.25 a b	3.25 ± 0.57 a b	3.3: 1
chlorothalonil	4.25 ± 0.25 a	4.25 ± 0.25 a	4.00 ± 0.50 a	4.3: 1
control	5.00 a	5.00 a	4.25 ± 0.28 a	4.7: 1

¹ Values in column with the same letter are not significantly different (P < 0.05, Tukey's test).

² Out of five larvae per replication (refer to Table 2).

DBM adult emergence at most concentrations ($P < 0.05$, t-test, $df = 6$) (Figure 2 A, B & C). Bt and chlorothalonil had no effect on D. insulare and DBM emergence from the treated pupae and results were not different from the control treatments ($P < 0.05$, t-test, $df = 6$) (Figure 2 D, E & F). However, other fungicides may have an effect. Maneb reduces the Microplitis croceipes (Cresson) emergence but not its host H. virescens (Felton & Dahlman 1984). The percent of D. insulare and DBM emergence from pupae in the control treatment were 90% and 95%, respectively.

D. insulare adult mortality 24 h after emergence from the insecticide-treated pupae was significantly higher in permethrin, azinphosmethyl and methomyl treatments than in Bt, chlorothalonil or controls ($P < 0.05$, Tukey's test, $F = 19.1, 12.5, 26.5$; $df = 5, 15$) (Table 5). This may be due to the effect of pesticide residues on the surface of the pupae or physiological effects from the pesticide that penetrated inside the pupae during the treatment. No mortality occurred on DBM adults 24 h after emergence from its insecticide treated pupae. This again indicated that DBM is generally insensitive to insecticides when compared to D. insulare.

D. insulare emergence from pupae treated with 1.0 mg AI/ml concentrations using the CO₂ hand held sprayer (Table 6) had a similar trend to emergence in the direct dip bioassay. As in direct dip bioassay, azinphosmethyl had less impact than permethrin and methomyl on the D. insulare emergence and was not different than the control treatments ($P < 0.05$, Tukey's test, $F = 38.4$, $df = 3, 5$). However, the percent of DBM emergence from pupae treated with insecticide was as high as in the control treatment. D. insulare adult emergence from treatments in the spraying bioassay was somewhat higher than in the direct dip bioassay. Probably more pesticide

Table 5. D. insulare mortality 24 h after emergence from pupae treated with insecticides in the direct dip bioassay.¹

Treatments	Concentration (mg AI/ml)		
	0.1	1.0	10.0
	% mortality ² (initial number) ± SE	% mortality ² (initial number) ± SE	% mortality ² (initial number) ± SE
permethrin	40 (10) ± 4.1 a	50 (2) ³ ± 3.5 a	– (0) ⁴
azinphosmethyl	38 (26) ± 2.9 a	25 (12) ± 2.5 a	35 (17) ± 2.0 a
methomyl	50 (26) ± 4.1 a	50 (12) ± 4.5 a	44 (9) ± 2.8 a
<u>B. thuringiensis</u>	5 (39) ± 2.8 b	9 (34) ± 0.8 b	12 (34) ± 0.8 b
chlorothalonil	7 (28) ± 2.8 b	12 (34) ± 1.2 b	16 (32) ± 2.4 b

¹ Diamondback moth mortality after emergence = 0% and D. insulare mortality = 6% (2/36) in the control.

² Values in column with the same letter are not significantly different (P < 0.05, Tukey's test).

³ Only two emerged from two of the four replicated.

⁴ No emergence.

Table 6. Diamondback moth and D. insulare adult emergence from pupae treated with 1.0 mg AI/ml pesticide solution using a CO₂ hand held sprayer.

Treatment	Diamondback moth (%, $\bar{x} \pm SE$) ¹	<u>D. insulare</u> (%, $\bar{x} \pm SE$) ¹
permethrin	95.0 ± 2.9 a	35.0 ± 2.9 c
azinphosmethyl	90.0 ± 4.1 a	77.5 ± 4.8 a
methomyl	90.0 ± 4.1 a	55.0 ± 2.9 b
control (water)	95.0 ± 2.9 a	90.0 ± 7.1 a

¹ Means in a column with the same letter are not significantly different (P < 0.05, Tukey's test).

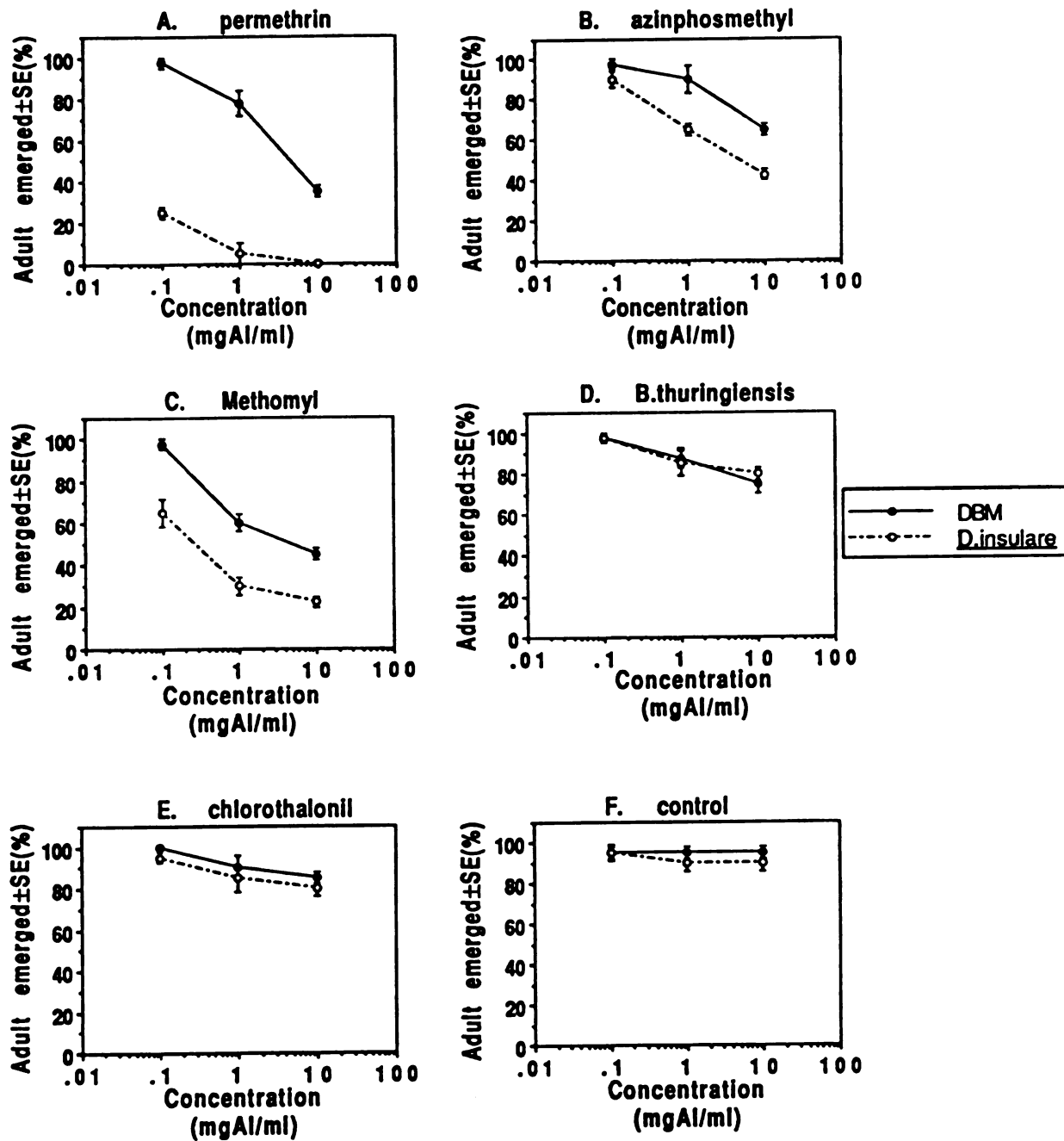


Figure 2. Percentage of *D. insulare* and diamondback moth adult emergence from insecticide treated pupae in the direct dip bioassay.

reached and affected the undeveloped D. insulare adult within the pupa in the direct dip than in the spraying bioassay. Percent of D. insulare adult emergence could be higher in the field because not all of the pesticide sprayed can reach the D. insulare pupae, as reported by Lasota & Kok (1986), Lim et al. (1986), and as discussed in Chapter 2.

Time of larval pupation and adult emergence. D. insulare parasitized DBM larvae took more time to pupate than non-parasitized larvae ($\bar{x}$ = 8.7 days vs 6.4 days, $P < 0.05$, t-test, $df = 3$; range = 8.3-9.0 days vs 6.0-6.4 days). DBM larvae parasitized by D. insulare feed at slower rates and probably need more time to get enough food before pupating than the non-parasitized larvae. This has been reported for other parasitoids (Parker & Pinnell 1973, Powel 1989). This suggests that the parasitized DBM larvae may consume less insecticide residue than non-parasitized DBM larvae because the residue decreases through time.

The mean days required by D. insulare adults to emerge from pupae were also significantly higher than for DBM ($\bar{x}$ = 8.8 days vs 5.3 days, $P < 0.05$, t-test, $df = 3$, range = 8.5-9.3 days vs 4.8-6.0 days, respectively). In view of this fact, D. insulare pupae may be exposed to insecticides longer than DBM pupae.

Conclusion

All of the insecticides tested were more detrimental to D. insulare than to DBM. However, the immature stages of D. insulare were less affected than the adults (Idris & Grafius 1991a, b). The fungicide chlorothalonil was apparently non-toxic to both D. insulare and DBM. Bt was the least harmful of the insecticides to D. insulare pupae and adults (Chapter 1), but still toxic to parasitized larvae if ingested. Low feeding rates of parasitized larvae

combining with the rapid degradation of Bt residues in the field (Soares & Quick 1990, Beckwith & Stelzer 1987) would minimize the impact of Bt treatment on parasitized DBM larvae.

Methomyl resistance in the DBM apparently protected the D. insulare within the parasitized larvae but D. insulare pupae were still vulnerable. The apparent mortality of D. insulare, and the survival of three DBM from parasitized larvae (Table 2), is of particular concern. Methomyl would not be chosen for control of resistant DBM, but might be used where the resistance status of the DBM was unknown or for control of other pests of cabbages.

Direct effect of pesticides such as Bt are low or non-existent (Chapter 1). However, in a truly integrated management program, indirect effects of pesticides on biocontrol agents need to be considered. A number of other factors also need to be considered. For example, parasitism of DBM also affects feeding and developmental rates of the larvae, which may alter treatment thresholds. The severe pest status and resistance of DBM to all insecticides require integration of biological control and other management tactics with pesticides, in spite of the complexities of the interactions.

OVERALL CONCLUSIONS

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Results of my studies indicated that none of the insecticides tested (except Bt and esfenvalerate to DBM larvae and adults respectively) in the laboratory bioassay and field experiments caused 100% DBM larval mortality. Larval mortality was higher through contact than ingestion. Bt had no impact on either D. insulare or DBM adults. Except for Bt, all the insecticides tested on DBM strains used in these studies had LC₅₀ values higher than the LC₅₀ values of the same insecticides tested on susceptible DBM larvae in Hawaii (Tabashnik et al. 1987). In the laboratory bioassay, the carbamate methomyl was the least toxic insecticide to both DBM larvae and adults but in the field thiodicarb, closely related to methomyl, was as effective as permethrin, esfenvalerate, azinphosmethyl and Bt products. Higher rates, different formulations and tank-mixing with other insecticides or sticker or adjuvant did not increase the insecticides' efficacy. Besides heavy rain (Wakisaka et al. 1990), behavioral resistance of DBM larvae toward insecticides as reported by Tabashnik (1986), Adams (1990), Hoy et al. (1990) and as mentioned in Chapter 1, may be another factor attributing to the low larval mortality. This confirms the reports of the incidence of scattered high resistant DBM populations in Michigan and other northern states of the United States (U.S.) to methomyl, permethrin and methamidophos by Shelton & Wyman (1990). The migration of DBM adult populations from the southern states of the U.S. (also had widespread DBM resistance) which, carried northward by favorable winds, temperature and humidity during the early spring or the second half of May each year, (Harcourt 1986) and the use of cabbage seedlings brought from those states (Shelton & Wyman 1990) may be the best explanation.

D. insulare adults were extremely sensitive to all pesticides (except Bt & chlorothalonil). Similar results also appeared in field cage experiments. D. insulare larvae within the parasitized host larvae were also indirectly affected by the pesticides through its host. Both parasitized and non-parasitized larvae were equally sensitive to insecticides through contact, but parasitized larvae were less sensitive through ingestion. This is because the parasitized larvae feed at a slower rate which enables them to consume the treated leaves with disintegrated insecticide residues. The joint action of D. insulare larvae that weaken their host and the insecticide ingested by the host caused the mortality of the parasitized and non-parasitized larvae not different 72 h after treatment. Of all the insecticides tested, Bt and azinphosmethyl were most toxic to parasitized DBM larvae if ingested. The percent of D. insulare adults emerged from insecticide treated pupae was not seriously affected except by permethrin at 10 mg AI/ml concentrations. However, the longer time needed by D. insulare adults to emerge from the pupae could expose them to more pesticides if frequent application is carried out. In the field, the percent of DBM parasitism by D. insulare was severely affected by the insecticide in field cages but not in field plot experiments. This was due to the abundance of the D. insulare population as a result of the presence of refuge plants around the field plot as reported by Harcourt (1986) and Sastrosiswojo & Sastrodiharjo (1986) than in the field cages. However, the level of parasitism varied and seldom reached 80%. The fungicide chlorothalonil had no impact on D. insulare and DBM.

From the above information it is clearly indicated that we need an integrated approach to control DBM. Using D. insulare will reduce pesticide use, environmental pollution and safety problems and slow down resistance development. Unfortunately, D. insulare alone cannot control DBM (Chapter

1). Therefore insecticides that are highly selective against DBM with low toxicity to D. insulare are still needed. Bt is the least harmful insecticide to D. insulare pupae and adults, but indirectly kills D. insulare larvae within the host larvae. Although the low feeding rate of the parasitized larvae and rapid degradation of Bt residues in the field would combine to minimize the impact of Bt on D. insulare, Bt also could not control DBM adults which determine the dispersion and dynamics of the population in the field (Tabashnik et al. 1987). In view of this fact, chemical insecticides are necessary to suppress DBM populations. Pyrethroids such as permethrin and esfenvalerate, or carbamates like thiodicarb which are highly toxic to D. insulare in the laboratory (Chapters 1 and 3), but are relatively safe to beneficials in the field (partly due to short persistency) (Powel et al. 1986, Ahmad et al. 1978, Crop & Whalon 1982, Iman et al. 1986, Farm Chemical Book 1990) could be used. These insecticides had no adverse effects on DBM parasitism by D. insulare in the field in my studies (Chapter 2). Their rate could be reduced without decreasing efficacy if they are tank-mixed with Bt (Iman et al. 1986) and of course this will favor D. insulare. Fungicides such as chlorothalonil (Grossman 1990) which had no impact on entomophagous fungi that attack DBM larvae and pupae (Wilding 1986) but also does not affect D. insulare (Chapters 1 and 3) should be used for controlling cabbage diseases. This would further increase the impact of the above-mentioned insecticides to DBM populations.

To achieve a maximum DBM mortality with minimum impact to D. insulare while delaying resistance development, the selected pesticides should be applied at the right action threshold level (ATL) related to marketability of the cabbage (Sastosiswojo & Sastrodiharjo 1986, Cartwright et al. 1990, Workman et al. 1981), late in the day, when high proportions of the

late DBM instar are at critical growth stages and the damage will significantly reduce the harvested yield (Shelton et al. 1982). This is very important especially to a large cabbage field which has terrace refuges for the parasitoid. The ATL could be increased because parasitized larvae feed at slower rates that will cause less damage over time than the non-parasitized larvae. However, regular monitoring of the parasitism level (larvae and pupae) in the field would have to be carried out. By doing so, it might help us to decide whether to spray immediately or follow a wait and see tactic.

Spraying insecticides, especially the ones that are less toxic to D. insulare pupae and adults when a high proportion of late instar DBM are parasitized by D. insulare may permit them to form D. insulare pupae and produce parasitoid adults while causing high mortality to the early instars that normally are less parasitized as reported by Garnett & Weseloh (1975) for gypsy moth larvae parasitized by A. melanoscelus (Ratzeburg). D. insulare adults emerging from the unaffected pupae could replace the field adult population but they might be killed by the indirect impact of pesticide residues on leaves. By knowing the age of D. insulare pupae, we could determine when and what type of pesticides should be used as well as how many D. insulare adults might be in the field after insecticide spraying. This is because D. insulare pupae are exposed to insecticides longer than the DBM pupae. To reduce the impact of pesticide residues on the leaf to the newly emerged parasitoid, the pesticides selected should have short or moderate persistency and the application should be carried out at the first half of the pupal period. This would allow the pesticides to disintegrate and the new parasitoid adults would not be contacting the lethal residues on the leaf. The newly emerged adult may also fly out of the field to find food and for mating and would not be contacted when they come back to parasitized DBM larvae.

However, the time they take to fly back to field to find hosts is of particular concern. The D. insulare adult is capable of laying eggs 24 h after emergence and it has a very high fecundity rate as well as excellent searching capacity (Bolter & Laing 1983). This may suggest that they would be back to the field to find their host 24 h after emergence.

The DBM adults emerge and are most active during the evening and lay their eggs at night (Chelliah & Srinivasan 1986). In contrast, D. insulare adults have a peak host searching activity from 9:00am-12:00am and relatively less in the afternoon (personal observation). M. croceipes (Cresson), the parasitoid of Heliothis sp. larvae, also have peak numbers in the field at a similar time (Powel et al. 1984). DBM adults have special physiological resistance mechanisms. That is, upon contact with insecticide residues, they automatize their metathorasic legs at the trochanter-femur joint (laboratory studies) (Tabashnik et al. 1988, Chen et al. 1985). On the other hand, high mortality parasitoids were attributed to the residues on the foliage (Powel & Scott 1986). Thus, it is clear that the DBM adult would be less affected by pesticide residues on the leaf than to D. insulare.

Direct impact of pesticides on the DBM adults is very important. Pesticide application during the late evening would be the best. By doing so, we could disrupt the egg oviposition activity of the DBM adult at night (Tabashnik and Mau 1986). At this time, most of the bigger larvae were also observed to feed on the upper surface of the leaves. They would be exposed to pesticides more and this will indirectly increase the pesticides' efficacy. D. insulare may also be less affected at this time.

Pesticides could also be applied on alternate rows rather than as a blanket spray (Casagrande & Haynes 1986). This would allow parasitized DBM larvae in the unsprayed rows to form D. insulare pupae. To get a high

rate of parasitism, we do not need too many parasitoid populations around. In fact, at high populations, the increase in percentage of parasitism will decrease due to mutual interference between host searching females (Bolter & Laing 1983). Although the DBM will attack the unsprayed rows they could be handled by D. insulare. We could also slow down resistant development by allowing the susceptible DBM from the unsprayed row to spread their genes to resistant populations. The unsprayed rows would be treated periodically to reduce injury. Alternate row spraying may be most applicable for a big acreage cabbage field where the parasitoids are most of the time present in the field and there are few refuge plants around.

Right timing, rotations and combinations of conventional pesticides are unlikely to retard resistant development in the diamondback moth due to cross-resistance and/or multiple resistance (Tabashnik et al. 1987). This again suggests that DBM management using biological control agents should be emphasized. Therefore, strategies that could enhance the capacity and maintain the D. insulare population in the field must be incorporated in the integrated DBM management program. In fact, biocontrol is much easier, cheaper and could reduce the problem of other pests of cabbage such as cabbage worm and cabbage looper. Such methods include planting trap crops, insectary plants, intercropping, resistant cabbage lines (glossy lines), modified overhead irrigation and removing the older cabbage leaves.

Planting trap crops such as wild mustard in two rows for every 25 rows of cabbages (Srinivasan & Krisna Moorthy 1990), in patches within or at the edge of the field, could reduce DBM populations on cabbage heads (Muniappan & Marutani 1990). Insectary plants grown along the edge or around the field would provide a good refuge for the parasitoids. The significance of these type of plants was observed in my field studies where D.

insulare parasitism was severely affected by pesticides in the field cage but not in the field plot experiments. Grossman (1990) reported that sunflowers used as insectary plants were the key element to integrating biological and chemical control of sweetpotato white fly at a 4060 ha tomato processing field in the Dominican Republic. Tomatoes intercropped in cabbage fields significantly reduced DBM damage (Talekar et al. 1986). Catherine & Tabashnik (1990) also reported that parasitism of Cotesia (= Apanteles) plutellae (Kurdjumov) was higher in cabbage interplanted with tomatoes than monospecific cabbage fields. Tomato plants produce a repellent that caused DBM adults to lay less eggs on cabbage leaves which eventually lowered the DBM larval population in the field. Resistant cabbage lines (glossy leaves) should be used because it could indirectly reduce the survival of the early DBM instar populations in the field (Eckenrode et al. 1986, Kimberly 1990, Eigenbrode & Shelton 1990). Sprinkler irrigation applied for five minutes at dusk on alternate days over the first three days, and every day thereafter, significantly reduced the DBM infestation and increased the yield over drip irrigation which received an equal amount of water (Talekar et al. 1986). The DBM population could be further reduced if the irrigation is carried out at night, which would disrupt the egg oviposition activity by the DBM adults (Tabashnik & Mau 1986). Removing old leaves might also reduce the availability of enemy-free space (EFS) and preferred pupation sites for DBM larvae. Reducing EFS will increase the efficiency of D. insulare and other parasitoid and predator searching. About 60% pupation occurred on the undersurface of the old leaves (Chelliah & Srinivasan 1986). This practice is very practical for small scale commercial cabbage growers.

Further studies on the impact of pesticides that are normally used in cabbage fields are necessary. This would enable us to select suitable pesticides

that best can be incorporated with D. insulare for integrated DBM management. The biology and population dynamics of D. insulare in the field should also be investigated in detail. This information could dictate the best timing of pesticide application and indirectly reduce pesticide usage. The positive effect of manipulating the environment by planting trap crops, insectary plants, intercropping, resistance lines, methods of irrigation, as well as removing older leaves should be demonstrated to the farmers as a means to extend these particular techniques better and faster. If all these techniques and methods are incorporated in an integrated DBM management program, it would not only overcome the DBM problem but also other pests of cabbages.

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APPENDIX 1

Record of Deposition of Voucher Specimens*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: 1991-7

Title of thesis or dissertation (or other research projects):

Impact of Pesticides on the Diamondback Moth, Plutella xylostella (L.)
and Effects on its Biological Control Agents, Diadegma insulare (Cresson)

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Other Museums:

None

Investigator's Name (s) (typed)

Idris Bin Abd.Ghani

Date Sept. 5, 1991

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America. Bull. Entomol. Soc. Amer. 24:141-42.

Deposit as follows:

- Original: Include as Appendix 1 in ribbon copy of thesis or dissertation.
- Copies: Included as Appendix 1 in copies of thesis or dissertation.
Museum(s) files.
Research project files.

This form is available from and the Voucher No. is assigned by the Curator, Michigan State University Entomology Museum.

APPENDIX 1.1

Voucher Specimen Data

Page 1 of 1 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of:							
		Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
<u>Plutella xylostella</u> (L.) Diamondback Moth			10			4	4		
<u>Diadegma insulare</u> (Cresson)						3	7		
<u>Diadromus subtilicornis</u> (Gravenhorst)						-	2		

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Idris Bin Abd-Ghani

Date September 5, 1991

Voucher No. 1991-7

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator

Date

14 Oct 91

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