

Interrelationship of *Bacillus thuringiensis* Berliner to Diamondback Moth,  
*Plutella xylostella* L. (Lepidoptera: Plutellidae), and Its Primary Parasitoid,  
*Diadegma insulare* Cress. (Hymenoptera: Ichneumonidae)

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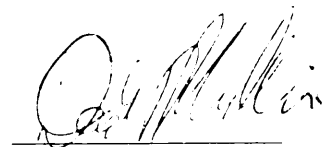
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Interrelationship of *Bacillus thuringiensis* Berliner to Diamondback Moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), and Its Primary Parasitoid, *Diadegma insulare* Cress. (Hymenoptera: Ichneumonidae)

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**Abstract**

The susceptibility of a population of diamondback moth, *Plutella xylostella* L (Lepidoptera: Plutellidae), collected from Montgomery County, Virginia, and its ability to develop resistance to *Bacillus thuringiensis* was evaluated. The field population of diamondback moth was found to be susceptible to *B. thuringiensis*. Selection pressure at 153 I.U. of *B. thuringiensis* endotoxin per ml for nine generations did not cause any significant difference in mean mortality of third instar diamondback moths although there was a trend towards lower mortality. There was significant negative linear correlation between generation and mean mortality of diamondback moth larvae ( $P = 0.003$ ,  $r^2 = 0.73$ ).  $LC_{50}$  increased from 264 I.U. of *B. thuringiensis* endotoxin per ml in generation I to 514 I.U./ml in generation IX

The interrelationship of *B. thuringiensis* to diamondback moth and its primary parasitoid, *Diadegma insulare* Cress (Hymenoptera: Ichneumonidae), was studied by determining: the differential response of third instar diamondback moth, parasitized and unparasitized, to *B. thuringiensis*; and the ability of *D. insulare* to discriminate between *B. thuringiensis*-treated and untreated hosts. There was no significant difference ( $P > 0.05$ ) between mean mortality of parasitized and unparasitized larvae at each of the three

concentrations consisting of 154, 334, and 2237 I.U. of *B. thuringiensis* endotoxin per ml. The regressions of the response of parasitized and unparasitized larvae, however, were highly significant ( $P = 0.0001$ ). The LC<sub>50</sub>s of parasitized versus unparasitized larvae were 373 and 176 I.U./ml *B. thuringiensis* endotoxin, respectively. Female *D. insulare* did not discriminate between *B. thuringiensis*-treated and untreated hosts. The percentage of *D. insulare* females emerging from *B. thuringiensis*-treated larvae (41.4%) was not significantly different from that of untreated larvae (32.0%).

Mean mortality of third instar diamondback moth subjected to *B. thuringiensis* endotoxin at 153, 334, and 2237 I.U./ml were not significantly different at temperatures of 15 and 20 °C, but were significantly lower than that at 30 °C. The effects of *B. thuringiensis* endotoxin residues on leaves under room conditions [ $27 \pm 1$  °C, RH  $27 \pm 7.2$  %, and 8 : 16 (L : D)] were not significantly different at 2 and 192 hours after treatment.

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## Chapter 1. Introduction

Cruciferous vegetables are economically important throughout the world and cultivation of *Brassica* crops have been practiced in Europe since ancient times. Since appearance is a determining criterion in marketing the crops, farmers have been relying exclusively on chemical insecticides to protect the yield and appearance of the market product from pests. This practice over the years has caused serious problems.

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is a cosmopolitan species with potential of becoming a serious pest of cruciferous crops worldwide. It has become one of the most difficult insects in the world to control because of its intrinsic biology and ecology, and wide range of host plants. The ability to migrate and disperse over long distances is one of the factors that makes this insect such a cosmopolitan species (Tabashnik and Mau 1986, Talekar and Shelton 1993).

Continued dependence and use of chemical insecticides for more than three decades has made the DBM resistant to all synthetic insecticides used because of cross resistance. Since DBM has become resistant to available insecticides, the lack of new insecticides, and increasing concern for the quality of the environment, viable alternatives to the regular use of synthetic insecticides are needed. Past experiences indicate that single-component strategies of DBM management will not be successful (Kadir 1992, Talekar and Shelton 1993). These concerns have stimulated research on alternate control measures.

The search for alternative controls includes evaluating the control strategies used before synthetic insecticides became the only powerful tool which was used to solve this pest problem. Since DBM is normally controlled by its natural enemies, especially parasitoids, introduction and conservation of its parasitoids should be a basic component

of integrated management of this insect. Therefore, the use of the insecticides which are less harmful to the natural enemies will be a requirement.

Of more than forty species listed as natural enemies of DBM, strong association mostly occurs with larval parasitoids from the genus *Diadegma* (Hymenoptera: Ichneumonidae). Parasitoids from the genus *Diadegma* are known to have the capability of keeping the population of DBM from causing serious damage. Some species have been reported showing tremendous success in controlling the DBM (Hardy 1938, Sastrosiswojo and Sastrodihardjo 1986; Talekar and Shelton 1993). Importation or conservation of parasitoids of similar potential is a worthwhile effort.

Microbial insecticides, e.g. *Bacillus thuringiensis* Berliner (Bt), have been used successfully, and are compatible insecticides with natural enemies. However, reports regarding resistance of several insects to Bt (McGaughey and Beeman 1988, Tabashnik et al. 1987 and 1990, Tanaka 1992) indicate that precautions are needed to maintain the efficacy of Bt. Therefore, whether DBM has developed resistance to Bt in a particular area, the stability of resistance, and the relationship of Bt to DBM and its parasitoids need to be studied.

The overall objectives of these studies are to determine:

1. Susceptibility of diamondback moth and its potential to develop resistance to *B. thuringiensis* under selection pressure.
2. Ability of *D. insulare* to discriminate between *B. thuringiensis* treated or untreated hosts.
3. Differential response of parasitized and unparasitized diamondback moth to *B. thuringiensis*.

## General Methodology

All of the experiments were carried out in the laboratory (insectary and greenhouse). Colonies of DBM and *Diadegma insulare* were collected from the Virginia Tech research farm (Whitethorne, Montgomery County, Virginia), and from an outbreak of DBM population in the greenhouse facilities at campus, during spring - mid summer 1992. The colonies were reared on potted cabbages in screened cages in the greenhouse. Diamondback moth and *Diadegma insulare* adults were fed on honey dropped on the top of the cages and misted with water. The *B. thuringiensis* formulation used was Dipel 2X, a commercially available wettable powder.

## Chapter 2. Review of literature

### Diamondback moth

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), has been reported as the most destructive pest of cruciferous crops from many parts of the world. Since crucifers have been cultivated for a very long time in Europe, DBM is considered of European origin. In Eastern Canada, DBM has been known for about a century. Harcourt (1955) and Moriuti (1986) summarized the synonymy of this insect since it was first described in 1746.

The host plants of this insect are restricted to members of the family Cruciferae that contain mustard oil and their glycosides. Harcourt (1986) found that DBM showed no preference between cultivated crucifers such as cabbage, cauliflower, brussel sprouts, and broccoli. Experiments by Gaines (1992), however, showed that DBM preferred broccoli to cabbage. Wakisaka et al. (1992) reported that DBM fed on a wild crucifer, *Capsella bursa pastoris*, had a lower reproductive ability than those fed on cultivated crucifers. However, wild crucifers still play an important role in maintaining DBM populations when cultivated ones are not available.

Reports on DBM as an important cruciferous crop pest from many different parts of the world (such as Europe by Hardy 1938, Eastern Canada by Harcourt 1986, Latin-American countries by Salinas 1986, Japan by Koshihara 1986, Northwestern Himalaya by Bhalla and Dubey 1986, Southeast Asia by Talekar and Shelton 1993) provide evidence that this insect can occupy widely different ecological conditions. This insect shows an unusual tolerance for a wide range environmental conditions. It seems probable that DBM can survive wherever *Brassica* crops are cultivated. Relative humidity has little or no direct effect on DBM development. Temperature is the main limiting abiotic factor with

the lower limit 10 °C and the upper limit 38 °C. The optimum temperature for oviposition is 20°C, but the optimum for all stages lies between 30 to 35 °C (Hardy 1938).

### **Life history**

The life cycle of DBM varies considerably with ecological and environmental conditions, especially temperature.

Egg. Eggs are minute, whitish yellow, cyclical to oblong, 0.5 mm in size. The eggs are laid generally singly or in groups of two to four on the underside of leaves, often along the mid-rib or principal veins and sometimes on the wall of the rearing jars. The egg incubation period ranges from 2.3 to 6.5 days (Harcourt 1957, Bhalla and Dubey 1986, Salinas 1986).

Larva. The newly hatched larva is whitish yellow to pale green, body dimensions on average measured 1.30 x 0.18 mm, with a pale brown head. First instars crawl to the lower surface of the leaves and bore through the epidermis and feed as leaf miners. At the end of the first instar, the larvae emerge from the mines, molt, and become surface feeders. The larvae undergo three molts resulting in four instars. Second and third instars feed with the head buried into the leaf tissue. Larvae usually feed from the lower surface, feed on all the leaf tissue but leave the upper epidermis and veins. Full grown larvae average 8 - 12 mm in length and are light green, moderately stout and smooth with short scattered hairs. When disturbed, larvae drop down suspending themselves by silken thread. Depending on temperature, larval duration can vary from 10 to 21.7 days (Harcourt 1955, Bhalla and Dubey 1986, Chelliah and Srinivasan 1986, Salinas 1986).

Pupa. The fully grown fourth instar wanders in search of a place to pupate. Most of the larvae pupate on the host plants on the basal third of the leaves. In the rearing cage,

some larvae climb the walls or ceiling and pupate there (Harcourt 1957). The larva constructs a loosely spun cocoon. The cocoon is firmly glued to the substratum where pupation occurs. Two days of quiescence marks the prepupal stage. The prepupa sheds its larval skin, and the cast skin remains attached to the caudal end of the pupa (Talekar and Shelton 1993).

The pupa is of the obtect type with average size 7 mm in length (Harcourt 1955). The newly formed pupa is yellowish green then turns brownish and becomes dark brown by the time of adult emergence. The pupal stage varies from 5 to 15 days with an average of 8.5 days (Harcourt 1957).

Adult. The moth is slender and grayish-brown with wing expanse about 14 mm (Bhalla and Dubey 1986, Chelliah and Srinivasan 1986). When folded, the wings display three diamond-shaped markings along the line where the wings meet (Borror et al 1989). The colors of the female are typically lighter, and the markings are less distinct (Harcourt 1955). DBM shows normal sex ratio of approximately 1:1 (52.9 : 47.1 by Harcourt 1960, 1.6: 1 and 1.6 : 1.4 by Bhalla and Dubey 1986).

### **Mating, Oviposition, and Fecundity**

Adult moths emerge primarily between 1:00 and 4:00 p.m. with a peak at 2:00 p.m. Mating begins at dusk on the day of emergence. Female mates only once while male mates as many as 3 times. Most females lay eggs on the same day of emergence. Oviposition begins in the evening and lasts up to 7:00 p.m. The oviposition extends to 10 days, with the most eggs laid on the day of emergence (Chelliah and Srinivasan 1986, Talekar and Shelton 1993).

The fecundity of females is affected by factors such as nutritional condition of the larvae, the nature of the host plants, the climatic conditions, the mating, and the presence

or absence of host plants on which to oviposit. More eggs are laid at low temperature and increased photoperiod (Chelliah and Srinivasan 1986). The number of eggs laid per female ranges from 18 to 356 with an average of 159 (Harcourt 1960).

### **Seasonal History**

Where food sources of DBM are available throughout the year, rapid turnover of generations occurs and overlap stages are found all year round (Talekar and Yang 1991). In India, 13 - 14 generations can be completed in a year (Chelliah and Srinivasan 1986). In Indonesia, where the life cycle can be completed in 20 -25 days, 15-18 generations occur per year (Ankersmit 1953). Harcourt (1957) reported 4 - 6 generations per year in Ottawa.

Hardy (1938) reported that DBM usually hibernate as adults, although a few pupae may survive the winter. In Canada, this insect does not overwinter. Migrants from Southern United States cause the annual infestation. The migration occurs in the spring aided by favorable northward wind. First generation breeds on cruciferous weeds, and subsequent generations attack cultivated crucifers (Harcourt 1986). It remains a controversial topic, however, whether DBM undergoes diapause or hibernates in any of its stages. If they can overwinter, resistant DBM may pass the resistant genes to the subsequent generation. Otherwise, the resistance genes will be lost in that area unless the next immigrants are resistant (Talekar and Shelton 1993).

### **Population Dynamics**

The population dynamics of DBM is strongly affected by many factors, environmental and mainly biotic (Harcourt 1960). An increased population density in the early season is triggered by favorable weather, high female density, and minimal activity of

*D. insulare* as a principal natural enemy in Eastern Canada (Harcourt 1986). Later in the season, the gradual decline in fecundity, hostile weather, and ability of natural enemies to catch up with the host cause a decrease in DBM population density (Wakisaka et al. 1992).

#### DBM ability to develop resistance

In the tropics, due to the availability of hosts practically throughout the year, rapid turnover under an ideal environment, and intensive use of insecticides, the DBM has now developed resistance to most chemical insecticides (Talekar and Yang 1991). The first case of DBM resistance was reported to DDT in 1953 from Indonesia. It took two years for the DBM to develop resistance in that area where 15 - 18 generations of that pest can be completed yearly (Ankersmit 1953). Resistance cases were reported decades later from other warm-weather areas (Talekar and Shelton 1993).

Resistance to diazinon, mevinphos, methomyl, carbofuran, pyrethrins, and DDT was reported from Taiwan (Sun et al. 1978). Liu et al. (1981) found that most field strains of *Plutella xylostella* in Taiwan developed resistance to the four major synthetic pyrethroids. Resistance to fenvalerate developed in four years, and in less than four years to permethrin, cypermethrin, and decamethrin. The result of studies conducted by Tabashnik et al. (1987) suggest that selection by insecticides other than pyrethroids, such as DDT and diazinon, cause cross resistance to permethrin. High level resistance of the DBM to different kinds of insecticides in Southeast Asia has elicited concern about continued successful crop protection (FAO 1979).

Insect growth regulators (IGRs) and pathogens offer promise as alternatives to broad spectrum insecticides which often have negative effects on natural enemies. However, resistance to IGRs has been reported from Thailand (Kobayashi et al. 1992)

The IGRs include chlorfluozuron, diflubenzuron, hexafluonuron, PH 70-23, NK-081 and NI-18 with various degrees of resistance. There was no recovery of sensitivity observed to this group of insecticides when no selection pressure was given for 40 generations. When treated with *B. thuringiensis*, it was found that the DBM was sensitive to the microbial insecticide.

Yu and Nguyen (1992) reported that DBM populations in North Florida showed extreme resistance to pyrethroids (resistance ranged from 2132 to 82,475 fold) followed by carbamates (405 and 409 fold for carbofuran and methomyl respectively), and moderate resistance to organophosphates (20 - 73 fold). No resistance to Bt subsp. *kurstaki* was observed in the field strain. The results of their studies revealed that the broad spectrum insecticide resistance was due to a multiple resistance mechanism including increased detoxication of insecticides by microsomal oxidase, glutathion transferase and reductases, and target site insensitivity (insensitive acetyl cholinesterase)

The DBM also has the tendency to autotomize legs shortly after tarsal contact with sublethal concentrations of fenvalerate residue. The moths that showed this tendency contained less insecticide at three hours after treatment, and survived better than the moths that do not drop legs (Moore et al. 1992). Treatment to either sex caused reduced and delayed oviposition. In the field, however, this disadvantage can be counteracted by faster recovery and higher survivorship of the autotomizing moths.

### *Bacillus thuringiensis*

With the development of insect resistance to synthetic chemical insecticides, microbial insecticides were considered as potential alternatives (Kissinger and McGaughey 1976). So far, microbial insecticides based on Bt Berliner are the most successful bioinsecticides. The discovery of Bt took place in the early part of the

twentieth century, however, its development for microbial control of pests was slow because of confusion between the different varieties (de Barjac 1981).

Bt Berliner is a spore-forming bacterium that produces a selectively toxic protein in the form of inclusion or crystal, that becomes the active component in Bt products. The inclusion consists of the protoxin form of one or more  $\alpha$  endotoxins. When ingested by susceptible hosts, the inclusion is solubilized and the protoxin is processed to the active  $\delta$  endotoxin form. The  $\delta$  endotoxin then binds to and destroys the midgut epithelium, causing rapid gut paralysis and termination of feeding (Soares and Quick 1992)

Bt is the most widely used and intensively studied microbial insecticide. It is highly toxic to certain pests, but not toxic to humans, most beneficial insects and most other non-target organisms. It also does not cause environmental and safety problems associated with synthetic insecticides (McGaughey 1985).

McGaughey (1978) studied the effectiveness of crystals and spores alone and the mixture of the two using *Plodia interpunctella* (Hubner) (Lepidoptera: Pyralidae) and *Ephestia cautella* (Lepidoptera: Pyralidae). The results showed that a mixture of 50 : 50 is the most toxic combination. Parasporal crystals were 30 times and 3 times more toxic than the spores to larvae of *E. cautella* and *P. interpunctella*, respectively.

Despite the merits furnished by Bt products, they are known to have a short residual activity under field conditions with inconsistent performance of Bt products in the field. This problem is due to the susceptibility to degradation of spore and naked crystals in conventional Bt product. A new formulation (MVP) improves Bt product characteristics by increasing foliar persistence and the use of selected toxins (Soares and Quick 1992).

MVP contains a selected  $\delta$  endotoxin of Bt subsp. *kurstaki* that is highly active against DBM. This toxin is encapsulated and stabilized within dead bacterial cells

Residual activity testing showed that MVP provided residual activity that was 2 to 3 times greater than products containing unprotected toxin crystals, and the persistence of MVP was 36 times greater than that of the conventional product when subjected to UV radiation. MVP also showed superior levels of control when compared with conventional Bt products and was equivalent or superior to standard chemicals (Soares and Quick 1992).

Results of efficacy studies by Chalfant (1992) of microbial and other insecticides to control crucifer pests showed that microbial insecticides (Bt Berliner products): Dipel, Javelin, Cutlass and MVP were moderately effective against *P. xylostella* and *Trichoplusia ni* (Lepidoptera: Noctuidae), but needed short (4-5 days) application intervals during severe pest pressure in the summer season in Georgia. Meanwhile fluobenzuron, flucycloxuron (molting inhibitors) and mevinphos (organophosphate) were still effective against DBM. However, esfenvalerate, bifenthrin and cyhalotrin were ineffective against the DBM.

#### Resistance to *Bacillus thuringiensis*

There was no report of resistance to Bt after more than two decades of its being widely used. It was thought that the complex mode of action of Bt made resistance unlikely to occur (Rossiter et al. 1990). However, selection pressure by intensive use of Bt ultimately altered the pathogen-host interaction. Report of insect resistance to Bt was first reported on stored product pests, the Indianmeal moth, *P. interpunctella*, and the almond moth, *Cadra* (= *Ephestia*) *cautella* (Walker) (McGaughey and Beeman 1988)

Resistance in field populations of DBM to Bt was first reported from Hawaii (Tabashnik et al. 1987 and 1990). Tanaka (1992) observed high level resistance of Bt Berliner in DBM in a watercress greenhouse in Osaka Prefecture in 1988. Jansson (1992)

also suspected that teflubenzuron and Bt induced resistance to field populations of DBM in Southern Florida since the efficacy of this product decreased over time.

Several studies have been carried out in attempts to understand the mechanisms involved in Bt resistance. Alkaline pH and proteolytic activity are required for activation of the toxin protein from the intact crystal (van Rie et al. 1989). Trypsin or trypsin-like proteases are required to convert Bt protoxin to active toxin (Witheley and Schnepf 1986). Schwartz et al. (1991) determined that changes in midgut pH or reduction in the proteolytic activation of the protoxins could serve development of resistance. MacIntosh et al. (1990) reasoned that protease inhibitor synergism with Bt may involve inhibition of protease that inactivate Bt or that degrade membrane-bound receptors of Bt

Johnson et al. (1990) found that there was no difference between the midgut of susceptible and resistance strains of *P. interpunctella* in their ability to activate Bt. The results of a study by Tabashnik et al (1992c) also indicated that altered proteases are not involved in DBM resistance to Bt.

Rossiter et al. (1990) stated that the variation in susceptibility of Gypsy moth to Bt was probably due to either differences in metabolic detoxification or differences in vigor. It may indicate the greater ability to repair a midgut damaged by toxin, or greater fat reserves that support the larvae through the starvation period associated with midgut paralysis following ingestion of Bt. In studying the susceptibility of a population of Indianmeal moth and Almond moth to Bt, Kissinger and McGaughey (1976) also concluded that the differences in susceptibility were related to population vigor rather than midgut pH.

Schwartz et al. (1991) determined reduced binding of the protein crystal to target sites on the midgut epithelium after crossing the peritrophic membranes as a prime mechanism for Bt resistance. Other possible mechanisms include increased degradation of

the toxin, reduced passage of the toxin through the peritrophic membrane, and reduced binding site concentrations in the midgut epithelium.

The mechanism that has been identified as a mechanism of resistance to Bt in Indianmeal moth (van Rie et al. 1989) and diamondback moth (Ferre et al. 1991) is reduced binding affinity of target site in the midgut. Experiments carried out by Schwartz et al. (1991) to determine the relative importance of behavioral versus biochemical mechanisms showed that the resistance mechanism is physiological (biochemical) rather than behavioral.

The characteristics of Bt resistance are evaluated in some studies. Hama (1992) noted that a high level of DBM resistance to tertiary amines and Bt was induced in the field by successive applications although development of the resistance was not fast. The resistance was rather unstable, but its stability tended to increase with resistance level. Tertiary amine resistance was more stable than Bt resistance. Extremely high level of Bt resistance decreased significantly in a few generations in the absence of insecticidal pressure. Thus, rotation of application of organophosphate insecticides, tertiary amines, and Bt is recommended. Different results were obtained by Tabashnik et al (1992a). Laboratory selection of DBM strain from the most resistant population showed increasing resistance 15 - 30 fold in nine generations. The resistance declined slowly in the absence of treatments. Therefore, rotation of this bioinsecticide with other insecticides is not an applicable tactic in managing resistance problem in DBM (Tabashnik et al. 1992b).

Tabashnik (1991), however, presumed that the results of resistance testing in the laboratory will not fully represent the field problem because of several reasons. Intensity of pressure is greater in the laboratory. There is substantial refuge from selection in the field because of limited persistence of Bt and spatial variation in concentration, occurrence of different stages in the field while only larva is affected by Bt, emigration, and different

types and amount of genetic variation within a population at the beginning of the experiment.

### Natural Enemies of DBM

Although all stages of DBM are attacked by numerous parasitoids and predators, larval parasitoids are the most predominant, effective, and highly studied. *Diadegma* and *Cotesia* (= *Apanteles*) (Hymenoptera: Braconidae) are the major genera known to be effective in suppressing DBM population. Genus *Diadegma* ( *Angitia*) is more common and the most significant numerically (Hardy 1938). *Diadromus* spp. (Hymenoptera: Ichneumonidae) pupal parasitoids, are found enhancing the effectiveness of natural enemies complex of DBM. The three genera are known to originate from Europe. Lack of the three genera causes intensive infestation of DBM in parts of the world where crucifers are cultivated intensively.

In Moldavia, Romania, Mustata (1992) reported that 62.9 % of 16,981 larval specimens collected during 1967 - 1972 were parasitized by a parasitoid complex of 28 species. Sixteen species of the parasitoids belong to the genus *Diadegma*. *D. fenestralis* showed the highest parasitization, followed by *D. armilata*, *D. crysosticta*, *D. vestigialis*, and *Diadromus subtilicornis*. Other species were reported to play a minor role.

Introduction of the genus *Diadegma* to New Zealand (Hardy 1938), Australia, Indonesia, and Taiwan (Talekar et al. 1992) has been successful in suppressing DBM. In Taiwan, parasitism of *D. semiclausum* was not high and early enough to completely control DBM population. Parasitization was 13.1 % after the initiation of parasitoid release and was 65.4 % after 10 weeks. However, the experiment indicated that *D. semiclausum* can infest DBM under Taiwanese field conditions, and has potential in controlling DBM. In one release area, 1,700 m above sea level, 3,500 parasitoid cocoons

per 17 ha were released two weeks after transplanting. Parasitization reached 75% and continued reducing DBM drastically. Releasing this parasitoid reduced the cost of insecticides from US\$ 1166.70 to US\$ 472.20 per ha.

Behavioral studies by Davis (1987) showed that releasing *Diadegma* that has been exposed to hosts on *Brassica* (=experienced) would make the augmentative technique more effective. Experienced females showed high efficiency in finding hosts. They also were attracted to damaged leaves, either artificially or by host. Davis further noticed that *D. eucerophaga* can be successfully integrated with chemical application because female *Diadegma* forages predominantly on the underside of leaves where insecticide coverage is low, even with electrostatic techniques. Reviewing the work done on DBM in Indonesia, Sastrodihardjo (1986) stated that *D. eucerophaga* might have developed a degree of resistance to insecticides since the parasitoid was well adapted to an environment intensively treated with insecticides.

Parasitoids from the genus *Diadegma* are polyphagous. Hardy (1938) reported that *D. cerophagus* and *D. fenestralis* have 8 and 24 hosts respectively, other than DBM. However, DBM is of primary importance to the parasitoids as is proven by the constant association reported from many parts of the world. Since DBM overwinters in the adult stage, the parasitoids must overwinter in hosts other than *Plutella*. Hyperparasitism of both species were 0.1%.

Knowing the environmental conditions favored by natural enemies is important for the natural enemies' establishment. A laboratory study showed that 15 - 20 °C is the optimum range of temperature for parasitism of DBM by *D. semiclausum* (Talekar and Yang 1991). Parasitization by this parasitoid is reduced sharply at temperatures approaching 30 °C.

Even from the same genus, *D. cerophagus* and *D. fenestralis* showed differential characteristics. Under the same conditions the colony of *D. fenestralis* gradually disappeared while the colony of *D. cerophagus* prospered. This phenomenon indicated that unfavorable environmental conditions for one species will not necessarily impair other species from the same genus (Hardy 1938). Legaspi (1986) also observed the differences between two broadly sympatric parasitoids, *D. eucerophaga* and *D. fenestralis*. *D. eucerophaga* showed a tendency to avoid superparasitism while *D. fenestralis* did not show this ability. Talekar and Yang (1991) reported that parasitism of *D. eucerophaga* was high at the temperature range of 15 - 25 ° C, and parasitism occurred only during photophase. No parasitism took place on the fourth instar of DBM.

Laboratory studies on the biology of *D. cerophaga* in Malaysia (Ooi 1980) revealed that longevity of male and female, respectively, were 40 and 73 days when fed on diluted honey. Fecundity of female, depending on the life span, averaged  $117 \pm 10$ . There were four instars with stadia depending on the age of hosts attacked. Unmated females produced male progeny. *D. cerophaga* is known to possess good characteristics as a biological control agent such as high searching capacity, is fairly host specific, has short developmental period and relatively high fecundity, and ability to occupy all the host habitat niches.

### *Diadegma insulare*

*Diadegma insulare* is a principal larval parasitoid of DBM in North America (Harcourt 1960, Bolter and Laing 1983, Latheef and Irwin 1983, Kok and McAvoy 1989, Fox and Eisenbach 1991, Alam 1992).

In Southwestern Virginia, *D. insulare* strongly influences DBM populations. In 1983 and 1984 the parasitoid caused 46 % and 69 % mortality of DBM respectively in

cabbage (Lasota and Kok 1986). Kok and McAvoy (1989) found *D. insulare* to be the most abundant of broccoli pest parasites which parasitized up to 37 % and 25 % DBM larvae in 1986 and 1987, respectively. Latheef and Irwin (1983) claimed that the presence of *D. insulare* in North America is a key factor that keeps DBM population from causing severe damage in crucifer cultivation. Biever et al. (1992) also noted that parasitism of DBM was consistently higher compared with two other major crucifer pests in North America: *T. ni* (Hubner) and *Artogeia (Pieris) rapae* (L.) (Lepidoptera: Pieridae).

In Coastal South Carolina, a complex of natural enemies was capable of maintaining DBM populations below economically important levels throughout the growing season. Predators accounted for up to 72 % of larval mortality. Parasitism by *D. insulare* in plots without the pyrethroid treatment reached 95 %. Reduction of the density of a complex of natural enemies by pyrethroid treatment caused DBM resurgence. DBM population levels in Bt treated plots were approximately the same as those in untreated plots. However, no resurgence occurred (Muckenfuss et al. 1992). In Jamaica, *D. insulare* remains active and exerts significant control in the field despite intensive use of chemical insecticides (Alam 1992).

*D. insulare* is known to attack all instars of DBM but prefers second and third instars for oviposition (Putnam 1968). In the laboratory at 20 °C the development of this parasitoid from egg to adult took 15.5 days when eggs were oviposited in second instar, and 9.5 days when the eggs were placed in fourth instar. One or two hours after parasitized DBM formed its cocoon, the parasitoid emerged from the host, then spun its own cocoon in 3 to 6 hours inside the host cocoon. The parasitoid then pushed the host remnants to the bottom of the host cocoon (Harcourt 1960).

Bolter and Laing (1983) observed that a female could parasitize 80 larvae per day at 25 °C. Longevity of the adult did not depend on food availability. Temperature,

however, affected the longevity and therefore also affected the fecundity of the female since *D. insulare* is a synovigenic species and has the ability to absorb the oocytes. The average fecundity at 17 °C was 595, increased to 814 at 23 °C, but decreased to 786 at 25 °C.

Both *D. insulare* female and male are attracted to cabbage plants. Damaged plants exaggerated searching behavior. *D. insulare* female has the ability to discriminate parasitized from unparasitized larvae. The discrimination probably relied on sensory stimulation of the ovipositor being inserted (Bolter and Laing 1983).

Comparative study showed the superiority of *D. insulare* to *Micropilitus plutella* (Hymenoptera: Braconidae). Maximum progeny obtained from a female parasitoid during a lifetime with regularly renewed supplies of hosts averaged 516 in *D. insulare* and 232 in *M. plutella* (Putnam 1968).

#### Impact of Bt on natural enemies

Salama et al. (1982) reported that *Micropilitus demolitor*, parasite of cotton leafworm, *Spodoptera littoralis* (Boisd.) fed on a diet containing Bt showed significant reduction in percentage of emergence and reproductive potential. Hamed (1979) studied the effect of Bt subsp. *kurstaki* on seven species of parasites and one species of predatory bugs of *Yponomeuta evonymellus* (Lepidoptera: Yponomeutidae). The studies showed that the hymenopterans *Diadegma armilata* (grav.), *Pimpla turionellae* (Boucher) (Lepidoptera: Ichneumonidae), *Agenoropsis fuscisollis* Dalm (Hymenoptera Encyrtidae) and *Tetrastichus evonymellae* (Bouche) (Hymenoptera: Eulophidae) were sensitive to Bt if they took up the spores of Bt with food. Food contaminated with *Bacillus* also affected a number of parasites. The interrelationship of a parasite of *Pieris rapae*, *Cotesia rubecula* (Hymenoptera: Braconidae), to Bt was studied by McDonald et al (1990). They

examined *C. rubecula* feeding on intoxicated hosts. The results showed that *C. rubecula* cannot successfully emerge after exposure to one-tenth the recommended field dosage, but can successfully emerge from feeding on lower dosages.

Conversely, some studies showed a synergism effect of Bt to parasitoids. Weseloh et al. (1983) reported that the percent parasitization of Gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), by *Apanteles melanoscelus* (Hymenoptera: Braconidae) was higher in plots treated with Bt than in untreated plots. The retarding effect of larval development caused by Bt on Gypsy moth provided the parasite with a large number of caterpillars. Similar results of laboratory experiments were obtained by Wallner et al. (1983). Gypsy moth larvae which were fed on artificial diet containing a sublethal dose of Bt showed longer developmental time of second instars up to 3 days. This prolonged stadium provided *Rogas lymantriae* Watanabe (Hymenoptera: Braconidae), a larval parasitoid that prefers second instars of less than 5 days, with more hosts. However, reducing *L. dispar* larval size by Bt provoked ovipositing *R. lymantriae* to deposit more unfertilized eggs, and therefore produced more male parasitoids.

By studying the differential toxicity of pesticides to *D. insulare* and its host, *P. xylostella*, Idris and Grafius (1993a) concluded that an integrated approach for control of DBM with Bt and *D. insulare* might permit control of DBM without directly affecting the parasitoid. Other insecticides tested: permethrin, azinphosmethyl, methomyl, and esfenvalerate, killed *D. insulare* adults at 1.0 mg AI/ml within 30 minutes.

**Chapter 3**  
**Susceptibility of Diamondback Moth**  
**and Its Potential to Develop Resistance to *Bacillus thuringiensis***  
**Under Selection Pressure**

## Introduction

*Bacillus thuringiensis* subsp. *kurstaki* has been widely used to control pest Lepidoptera for more than two decades. It was presumed that resistance to Bt was unlikely to occur due to its complex mode of action. Until recently no report documented such an eventuality (Rossiter et al. 1990). However, laboratory studies conducted by McGaughey and Beeman (1988) showed that resistance in five colonies of Indian meal moth, *Plodia interpunctella* (Hubner), increased from 2 to 29 fold within three generations and from 15 to 100 fold in approximately 40 generations under relatively low selection pressure. Resistance in one colony increased more than 250 fold with higher selection pressure. In the almond moth, *Cadra cautella* Walker, colony resistance increased approximately 7 fold in 21 generations.

Recently, Tabashnik et al. (1990) were the first to report that a field population of diamondback moth developed resistance to Bt. Mortality was 56 - 65 percent lower in the resistant population than in those of two susceptible laboratory colonies.

The most important factor influencing the rate of development of resistance is the intensity of selection pressure that selects for phenotypes caused by one or more genes (FAO 1979). In Virginia, where crucifers are not planted throughout the year and Bt has not been extensively used, the probability of resistance to Bt is low. To prolong the effectiveness of Bt it is important to determine the susceptibility of diamondback moth and its potential to develop resistance before it occurs. The objective of this study is to evaluate the susceptibility of a field population of DBM and its ability to develop resistance to Bt.

## Materials and Methods

The microbial insecticide used was *Bacillus thuringiensis* subsp. *kurstaki* in formulation Dipel 2X containing 32,000 International Units (I.U.) of potency per mg and recommended rate for cabbage approximately 0.90 gram per liter.

Bioassays were conducted using the leaf-dip method. Two young cabbage leaves (about 8.0 cm width) were dipped in each concentration of Bt for 30 seconds and left to dry for about 2 hours. For control, leaves were dipped in water. The stems of the leaves were inserted through a hole of an inverted plastic container lid (12 cm in diameter) in 200 ml jar of water. A ventilated plastic container of 900 ml was used to cover the leaves after larvae were placed on the leaves. About 10 third instar DBM were used for an experimental unit. The experiment was carried out in the insectary under room conditions maintained at 80 °F (26.7 °C). Larval mortality was recorded daily until adult emergence or death of larvae.

Preliminary tests using a leaf-dip method were performed to determine LC<sub>30</sub>, LC<sub>50</sub> and LC<sub>90</sub>. Larvae were tested from diamondback moth colonies established in the insectary a few months after field collection. At least 500 diamondback moth (larvae, pupae, and adults) were collected from two locations. Concentrations equivalent to LC<sub>30</sub>, LC<sub>50</sub>, LC<sub>90</sub>, and a control, or 153, 334, 2237 I.U. of Bt potency per ml respectively, plus control, were used for subsequent tests.

The first colonies tested with the above concentrations were considered as generation I. Emerging adults from the replicates of each treatment were caged together to mate. The offspring were tested at the same Bt concentrations as the previous one(s) for several generations. At the end of the experiment, the latest generation was treated with Bt at concentrations 153, 334, 2237 I.U. of Bt potency per ml, and control.

Mortality data were corrected against control mortality using Abbott's formula (Abbott 1925). Mortality from each generation was compared to determine the susceptibility of the colonies. Data were analyzed using ANOVA (Hintze 1990). Probit analysis was used to compare LC<sub>50</sub>s of the initial and the final experiment

### Results and Discussion

All test larvae were confined on treated leaves throughout their development. It is unlikely, therefore, that survivability was due to behavioral escape. Since the amount of Bt consumed increased with time, eventually most larvae would acquire a cumulative amount which was lethal to a susceptible individual

Data of this experiment revealed that field populations of DBM in Virginia are still susceptible to Bt. Bt treatment at concentration 153 I U up to nine generations did not cause any significant differences in larval mortality ( $P = 0.1129$ ,  $F$  value = 1.68,  $df = 9$ ), while laboratory selection by Tabashnik et al (1992a) on a field resistant population for nine generations increased resistance 15 - 30 fold. Mean mortality, however, showed a decline with increased selection (Table 2.) and (Fig. 1.). There was significant negative correlation between generation and mean mortality of DBM larvae ( $P = 0.003$ ,  $r^2 = 0.73$ ) The initial experiment resulted in LC<sub>50</sub> being 264 I U Bt potency, which is approximately equivalent to one-hundredth of the field recommended dose. Selection up to nine generations increased the LC<sub>50</sub> to 514 I.U. (Table 1.). Although the reduced mean mortality with generation might be partly due to increased adaptability of the colonies to the rearing method, the increased LC<sub>50</sub> and reduced mortality means could be warning signals for the possible development of resistance under selection pressure

Bt treatment at higher concentrations (334 and 2237 I U/ml) produced less progeny. Besides fewer survivors at the higher concentrations, the effect of Bt could also

cause reduction in fecundity of the survivors. Sneh and Schuster (1989) indicated that surviving individuals had a lower rate of development. While treatments at 153 I.U. Bt endotoxin per ml were able to be carried out up to nine generations during the period of experiment, treatments at 334 and 2237 I.U./ml only reached four and two generations, respectively.

There was no significant difference in mean mortality of third instar DBM treated with Bt at 334 I.U./ml up to four generations ( $P = 0.235$ ,  $F$  value = 1.49,  $df = 3$ ). Treatments at 2237 I.U. Bt endotoxin per ml did not cause any significant difference in mean mortality between first and second generation ( $P = 0.862$ ,  $df = 21$ ,  $t$  value = - 0.176)

Hama (1992) reported that a high level of DBM resistance to Bt was induced in the field by successive applications, and the development of the resistance was not fast. Since crucifers are not cultivated throughout the year in Virginia, Bt resistance in DBM in this area is unlikely to occur in the near future

There are reports indicating that resistance of lepidopterous pests to Bt is not yet widespread. In Florida, while the diamondback moth populations showed resistance to a broad spectrum of chemical insecticides, no resistance to Bt subsp. *kurstaki* was observed (Yu and Nguyen 1992). Studying the susceptibility of DBM from different places, Shelton et al. (1993) reported that a population from Indonesia was very susceptible to Bt products compared with DBM populations from six states in the United States.

Results of experiments by Sneh and Schuster (1983) on 10 generations of *Spodoptera littoralis*, from individuals that had survived feeding as larvae on Bt, did not show increased resistance. On the contrary, the subsequent generations showed a higher susceptibility to the Bt toxin than corresponding generations of untreated larvae. They also noted that the development of those larvae (25 - 36 days from hatching to pupation) was considerably slower than that of untreated ones (18 days).

Field resistance of DBM to Bt was first reported from Hawaii (Tabashnik et al. 1990), where the frequency of use of Bt in this area in controlling DBM populations has been equivalent to the excessive use of conventional insecticides (Tabashnik et al. 1992a). However, even though in most areas DBM populations are found still susceptible to Bt, precautions need to be taken to prolong the effectiveness of Bt.

Resistance is a phenomenon that typically develops rapidly. Neglecting the warning of its occurrence can cause a big loss within only a brief period after first detection (National Research Council 1986). This warning has to be taken more seriously regarding the intrinsic characteristics of DBM, such as their ability to migrate over long distances.

Tabashnik et al. (1992b) suggested that resistance management of DBM to Bt should be done before it occurs since the susceptibility is not restored rapidly in the absence of treatment, and the use of mixtures of Bt toxin will not halt resistance development. On the contrary, laboratory selection of strains that had developed 30 fold resistance in the field could increase their resistance level to more than 700 fold.

To prevent the occurrence of resistant individuals, Sneh and Schuster (1983) suggested a 'Biologically Controlled System' by establishing a broad variety of natural enemies in an environment. Implementation of a variety of biological as well as integrated control strategies should be able to reduce the selective pressure of Bt on pests (Shelton et al. 1993).

Table 1. Response of DBM colonies to Bt at initial and final<sup>a</sup> experiments

Experiment	n <sup>b</sup>	LC <sub>50</sub> <sup>c</sup>	Intercept	Slope ± Std. error	95 % Conf. interval
Initial	968	264	1.92	1.271 ± 0.111	75.7 - 918.2
Final	224	514	0.12	1.801 ± 0.249	79.1 - 3339.2

<sup>a</sup> after nine generations

<sup>b</sup> total larvae.

<sup>c</sup> I.C<sub>50</sub> is expressed in International Units of *B. thuringiensis* potency per ml.

Table 2. Percent corrected mean mortality of third instar DBM to Bt for nine generations.

Generation	n <sup>a</sup>	% corr. mean mortality ± Std. error
I	262	39.8 ± 4.8
II	419	34.2 ± 4.6
III	156	37.4 ± 6.3
IV	152	37.3 ± 6.0
V	189	25.1 ± 6.3
VI	93	35.7 ± 7.5
VII	131	25.7 ± 6.6
VIII	84	21.0 ± 8.1
IX	103	19.9 ± 6.3

<sup>a</sup> number of larvae used

Regression equation between generation and mean mortality  $y = 42.7 - 2.4x$   
 (y = % corrected mean mortality, x = generation),  $P = 0.003$ ,  $r^2 = 0.73$

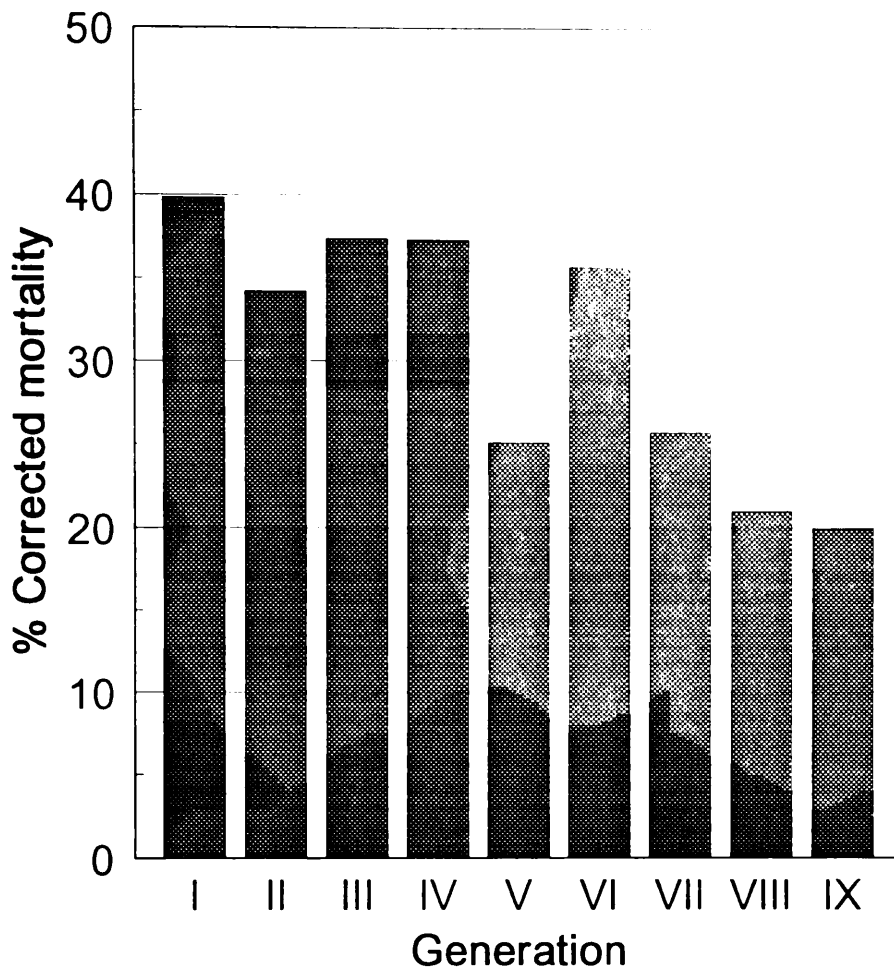


Fig. 1. Mean mortality of third instar DBM for nine generations subjected to Bt at 153 I.U./ml

## Chapter 4

### **Differential Response of Diamondback Moth, Parasitized and Unparasitized by *Diadegma insulare*, to *Bacillus thuringiensis***

## Introduction

The use of Bt as a 'safe' insecticide to non-target organisms has made it an interesting study for many researchers. This interest generated much information on responses of predators and parasitoids to Bt. Some evidence suggests that Bt may induce mortality in beneficial non-target arthropods, but it is not clear whether the mortality was directly induced by the Bt toxin or indirectly caused by deteriorating quality of available food source of the parasitoid larvae.

McDonald et al. (1990) showed that fourth instar *Pieris rapae* parasitized by *Cotesia rubecula* are less susceptible to low dosages of Bt than unparasitized larvae. Studies by Muck et al. (1981) on effects of Bt on *Apanteles glomeratus* and *Pimpla turionella* showed that Bt had no adverse effect on the parasitoids even when Bt was taken orally. Idris and Grafius (1993a) indicated that Bt had no adverse impact on *Diadegma* adults even at high concentrations, whereas other chemical insecticides tested caused significant mortality. These results suggest that deleterious effects of Bt on Hymenopterous parasitoids are unlikely.

Parasitized larvae may escape from exposure to Bt. Parasitoid larvae may alter the behavior of their hosts making them less likely to consume the *Bacillus* (Flexner et al. 1986). Proper timing of Bt application and *Diadegma* release, e.g. releasing *Diadegma* prior to Bt application, may enhance the compatibility of Bt and the parasitoid if there is a differential response between DBM parasitized and unparasitized larvae to Bt. The objective of this study is to investigate whether there is a differential response to Bt by third instar DBM parasitized by *Diadegma* versus unparasitized larvae.

## Materials and Methods

Colonies of diamondback moth and *D. insulare* were maintained at the insectary facilities of VPI & SU, Blacksburg. To obtain parasitized and unparasitized larvae at approximately the same age, half of the potted cabbages that had been exposed to DBM colony for oviposition were placed in cages with *D. insulare* females and another half of the potted cabbages were kept in cages without the parasitoids.

Concentrations of Bt used were equivalent to concentrations of LC<sub>30</sub>, LC<sub>50</sub>, and LC<sub>90</sub> obtained from a preliminary study, or 153, 334, and 2237 International Units of *B. thuringiensis* endotoxin per ml, plus a control.

Bioassays were conducted using a leaf-dip method. About 10 third instar DBM were used per replication. The test was conducted at  $26.7 \pm 1$  °C. Six replications were performed for either parasitized and unparasitized larvae. They were checked every day until adult emergence or death of larvae.

Mortality data for each treatment were corrected against mortality of control using Abbott's formula (Abbott 1925). Probit analysis was used to calculate LC<sub>50</sub>s for treatments of parasitized and unparasitized larvae. Parallel Line Analysis (PLA) (Finney 1979) was used to compare the differential dosages for certain mortality level in parasitized and unparasitized treatment. The means of treatments were analyzed using ANOVA (SAS Institute 1990).

## Results and Discussion

Although the mean mortality of parasitized larvae was generally lower than that of unparasitized larvae (Fig. 2), Tukey's multiple comparison reveals no significant statistical differences between mortality of parasitized and unparasitized larvae at each of the three treatment levels (LSD value = 89.2, n = 275, Alpha = 0.05) (Table 3). LC<sub>50</sub>s of

parasitized and unparasitized larvae obtained from Probit Analysis were 372.7 and 174.5 International Units, respectively. Parallel Line Analysis, however, reveals that the regression lines of the response of parasitized and unparasitized larvae to Bt are highly significant ( $P = 0.0001$ ,  $F$  value = 27.8). This indicates that parasitized larvae were less susceptible to Bt. Similar results were reported by McDonald et al. (1990) where *Pieris rapae* larvae parasitized by *Cotesia rubecula* were less susceptible to low dosages of Bt than the unparasitized ones.

Idris and Grafius (1993b) reported different results of a similar experiment. They found no significant differences between mortality of DBM larvae parasitized by *D. insulare* and unparasitized larvae subjected to Bt. They presumed, however, that the lack of mortality differences between parasitized and unparasitized larvae in laboratory experiments was due to the joint action of the cumulative effect of the toxin consumed and the feebleness of the parasitized larvae due to the parasitism. They observed that Bt did not cause any significant difference in the amount of food consumed by parasitized larvae and unparasitized larvae, whereas other pesticides tested caused parasitized larvae to feed less. The parasitized larvae, however, showed a lower feeding rate than the unparasitized larvae, and thus, reduced mortality of parasitized larvae. Idris and Grafius (1993b) concluded that pesticides had no adverse effect on immature stages of *D. insulare* within the host body. They also observed that more female parasitoids were found in Bt and other pesticide treatments except chlorothalonil. This is highly beneficial for integrated management.

Since parasitized larvae show a lower feeding rate than unparasitized larvae (Idris and Grafius 1993b), and Bt has short foliage persistence in the field (Beckwith and Stelzer 1987, Soares and Quick 1992) the immature parasitoids are able to complete their development before the hosts consume a lethal amount of Bt. This interaction suggests

high compatibility of *Diadegma* and Bt in the field since the larvae that escape from parasitism are expected to acquire a lethal amount of Bt toxins. Flexner et al 1986 stated that for Integrated Pest Management, when *Diadegma* release is combined together with Bt application, application of Bt after parasitoids have infested the hosts would greatly reduce indirect mortality of the beneficial insects.

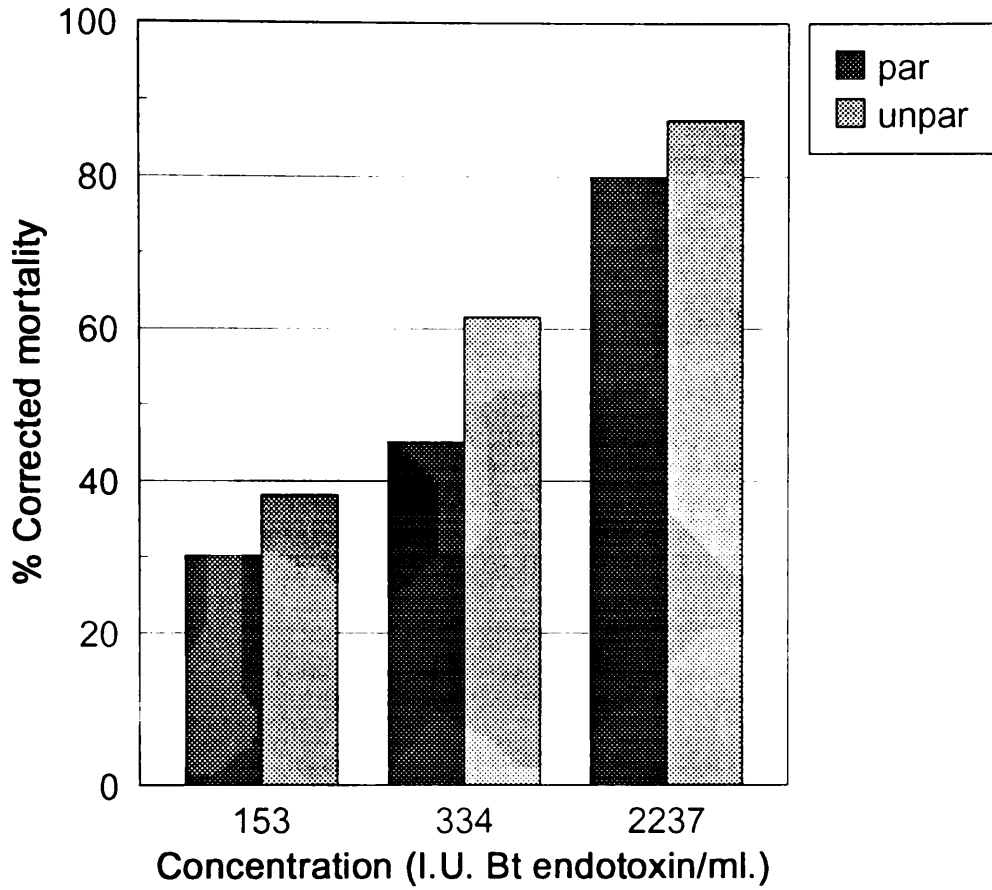


Fig. 2. Mean mortality of parasitized and unparasitized third instar DBM at different concentrations of Bt.

Table 3. Mean mortality of parasitized and unparasitized larvae to Bt at three different concentrations<sup>a</sup>

Concentration <sup>b</sup>	% Mortality (mean $\pm$ SE)		Corrected % Mortality (Mean $\pm$ SE) <sup>c</sup>	
	Parasitized	Unparasitized	Parasitized	Unparasitized
Control	16.1 $\pm$ 4.2	6.8 $\pm$ 3.3	0	0
153	42.8 $\pm$ 8.5	42.3 $\pm$ 5.9	30.2 $\pm$ 12.2cd	38.1 $\pm$ 5.5cd
334	54.8 $\pm$ 10.4	64.0 $\pm$ 6.3	45.1 $\pm$ 13.4bcd	61.6 $\pm$ 5.9abc
2237	84.3 $\pm$ 5.6	88.2 $\pm$ 4.3	80.0 $\pm$ 7.8ab	87.5 $\pm$ 4.7a

<sup>a</sup> Mean of 6 replicates (n= 55 to 79)

<sup>b</sup> expressed in International Units of Bt potency per ml

<sup>c</sup> Means having the same letter in each row or column are not significantly different at the 0.05 level, Tukey's test (Zar 1984)

## **Chapter 5**

### **Ability of *Diadegma insulare* to Discriminate Between *Bacillus thuringiensis*-Treated and Untreated Hosts**

## Introduction

Ichneumonids of the genus *Diadegma* are known to be effective natural enemies of the diamondback moth in many parts of the world. Even though there is a report that shows *Diadegma insulare* controlling a diamondback moth population in the field where chemical insecticides are intensively applied (Alam 1992), in general however, the detrimental effect of chemical insecticides reduces the effectiveness of *Diadegma* spp. (Muckenfuss et al. 1992, Idris and Grafius 1993a). *Bacillus thuringiensis* which maybe a safe insecticide for natural enemies is recommended by some researchers to be combined with the release of *Diadegma* (Talekar et al. 1992, Talekar and Shelton 1993).

Several studies indicated that Bt does not cause significant mortality to adult parasitoids by direct contact (Flexner et al 1986, Idris and Grafius 1993a). The effects of Bt on immature stages of *Diadegma insulare* were studied by Idris and Grafius (1993b). They stated that there was no difference in susceptibility between parasitized and unparasitized DBM larvae to Bt. It is still not clear to what extent Bt directly affects the immature stage of the parasitoid.

Parasitism of Bt-treated larvae might cause mortality to the parasitoids either directly or indirectly. Salama et al (1982) reported that *Microplitis demolitor*, parasitizing cotton leafworm, *Spodoptera littoralis* (Boisd.), fed on a diet containing Bt showed significant reduction in percentage of emergence and reproductive potential. Conversely, some studies showed synergistic effects of Bt on parasitoids. Weseloh et al (1993) reported that the percentage parasitization of Gypsy moth by *Apanteles melanoscelus* was higher in plots treated with Bt than that in untreated plots. The retarding effect of larval development caused by Bt in Gypsy moth provided the parasite with a larger number of host caterpillars.

It is difficult to quantify the exact amount of Bt consumed by larval parasitoids (Flexner et al 1986). Even if the immature stages of the parasitoids consume a certain amount of Bt toxins, as long as the parasitoid gut conditions are not suitable for activating the toxin, there would not be a direct effect of Bt on the parasitoid larvae. However, indirect mortality caused by deteriorated quality of the food source (infested host) could be a major factor of parasitoid mortality.

The ability of hymenopterous parasitoids to discriminate between Bt infected and uninfected hosts can be a mechanism by which adult parasitoids can avoid direct and indirect mortality of their progeny and therefore increase the compatibility of Bt and the natural enemies. The objective of this study is to investigate whether *Diadegma insulare* has the ability to differentiate between Bt-treated and untreated larvae of the diamondback moth.

### Materials and methods

A number of early third instar DBM were fed on cabbage leaves that had been treated with Bt at a concentration equivalent to LC<sub>90</sub> obtained from a preliminary test or 2237 International Units of *B. thuringiensis* per ml, for 24 hours. For control, the same age larvae were fed on untreated leaves. The treated larvae and control were exposed together to *D. insulare*.

Approximately ten larvae were used per unit experiment, replicated 10 times. For easy observation, larvae were given a dot mark on their backs (dorsoabdomen) with a marker. To investigate the marking effect, half of the treated larvae were marked and exposed together with the unmarked control. Another half of the treated larvae was left unmarked and was exposed together with the marked control. Each experimental unit was exposed to a female parasitoid for about 3 hours.

After being exposed to the parasitoid, treated larvae were separated from the control. Mortality and adult emergence were recorded daily. Percent parasitization was based on the emerging adults.

A *t*-test analysis was used to evaluate the ability of *Diadegma* to discriminate between Bt-treated and untreated hosts. The same analysis was used to determine the artificial marking effect on parasitism. The proportions of female parasitoids emerging from treated and untreated larvae were compared using z-test analysis.

### Results and Discussion

Parasitism of Bt-treated and untreated DBM is shown in Table 4. In this study, percent parasitization was based on the total number of emerging adults (*Diadegma* and DBM). Parasitism of non-surviving larvae (larvae died because of Bt treatment) was not included.

Analysis of Variance from the treatments showed no significant difference ( $P=0.874$ ,  $F$  value = 0.23,  $df = 3$ ). *t*-test analysis of pooled data (marked and unmarked) between Bt-treated and untreated larvae revealed no significant difference ( $P=0.454$ ,  $df = 38$ ,  $t$  value = 2.649E-04). To evaluate marking effects on parasitism, data were pooled from Bt-treated and untreated treatments and analyzed using *t*-test analysis. No significant difference was found from the analysis ( $P=0.586$ ,  $df = 38$ ,  $t$  value = 0.432).

These results indicated that *D. insulare* did oviposit on Bt-treated larvae of DBM. However, oviposition was only observed from the Bt-treated larvae which were able to survive the treatment. It is not clear from this experiment whether the parasitism of Bt-treated larvae was due to the incapability of *D. insulare* females to differentiate between Bt-treated and untreated larvae, or on the contrary, due to the ability of the female parasitoid to estimate the suitability of the host to support the development of its progeny.

Further study is needed to obtain information on whether or not *D. insulare* is able to avoid ovipositing on Bt-treated larvae that eventually die, and therefore be unable to support full development of the parasitoid.

The data also suggested that artificial marking had no effect on parasitization. This result supports the hypothesis of Bolter and Laing (1983) that the ability of *D. insulare* females to discriminate parasitized from unparasitized hosts relied on sensory stimulation of the ovipositor being inserted rather than visual stimulation.

From the total adult emergence of 193, 68.9% were *D. insulare* (Table 5). Of emerging adult parasitoids, 36.1 % were female. Thirty two percent of the parasitoid adults emerging from untreated-larvae treatments were female, while 41.4 % female parasitoids were recorded from Bt-treated larvae treatments. *z*-test, however, revealed no significant difference in the proportion of emerging female parasitoids from Bt-treated larvae ( $P = .2640$ ,  $z$  value = 1.1168). If we assume that Bt treatment killed DBM larvae containing the same proportion of female and male parasitoids, these data show disagreement with either the results of Idris and Grafius's (1993b) where more female *D. insulare* were obtained from DBM larvae surviving Bt and other insecticide treatments; or the results of Wallner et al.'s(1983), where reduced size of *Lymantria dispar* larvae treated with Bt caused *Rogas Lymantriae* to oviposit more unfertilized eggs, therefore producing more male parasitoids.

Once a parasitoid successfully emerges from an infested host, there is still the possibility of the parasitoid being affected by either a direct or indirect sublethal dose of Bt. Sublethal effects of pesticides on natural enemies that have been documented include the following: reduced egg production, reduced host consumption, reduced percent parasitism, reduced adult longevity, increased parasitoid developmental time, and skewed sex ratio (Flexner et al. 1986). Further studies on the performance of surviving adult

parasitoids would give valuable information on the population dynamics of *Diadegma insulare*.

Table 4. Mean<sup>a</sup> percent parasitization of Bt-treated and untreated DBM larvae by *Diadegma insulare*.

Treatment	n <sup>b</sup>	Mean $\pm$ SE	
		Marked	Unmarked
Treated	123	68.9 $\pm$ 10.8	73.0 $\pm$ 9.6
Untreated	119	78.1 $\pm$ 9.6	70.5 $\pm$ 7.2

<sup>a</sup> Mean of 10 replications

<sup>b</sup> Number of larvae used.

Table 5. Ability of *D. insulare* to discriminate between Bt-treated and untreated hosts.

Factor	Treated		Untreated		Total <sup>a</sup>
	Marked	Unmarked	Marked	Unmarked	
Larvae used	61	62	60	59	242
Emerging adult	43	43	51	56	193
DBM	13	15	13	19	60
<i>D. insulare</i> (M)	19	15	23	26	133
<i>D. insulare</i> (F)	11	13	15	9	48

<sup>a</sup> Total number from 10 replications.

## Chapter 6

### **Residual Activity of *Bacillus thuringiensis* and Effect of Temperature on Its Efficacy**

## Introduction

Microbial insecticides derived from *Bacillus thuringiensis* Berliner are the most successful bioinsecticides so far. It is known to be a selective insecticide that is potentially effective in controlling lepidopterous pest targets. Yet, despite their advantages, there are some limitations of the Bt products. Since Bt must be ingested to be effective, foliar persistence is essential to achieve good efficacy in the field. Otherwise, larvae would get a chance to recover before consuming lethal amounts of the toxins. Residual studies in the field, however, showed that Bt has short residual activity (van Frankenhuyen and Nystrom 1989). Lack of foliar persistence is the most important factor causing inconsistent performance in the field, and is responsible for Bt not being more widely used. It is also very difficult to evaluate the efficacy of Bt, because, even from the same product, its performance may vary in the field (Soares and Quick 1992).

This implies that without degrading factors in the field environment, Bt would perform better. Therefore, enhancing the performance of Bt formulations would increase the effectiveness in the field. Residual activity testing under laboratory conditions should help to explain the potential efficacy of a product. By modifying each factor that possibly reduces the effectiveness of Bt products in the field, we may be able to find out the key factor responsible for failure under field conditions.

Some researchers recommend the use of Bt and manipulation of parasitoids, *Diadegma* spp., for integrated pest management of the diamondback moth (Talekar et al. 1992, Talekar and Shelton 1993). Results of several studies confirmed the compatibility of these two control agents. To obtain good compatibility, at least one strategy or control agent must be effective under a circumstance which is unfavorable for other strategies or control agents.

Following the introduction of *Diadegma semiclausum* to Taiwan (Talekar et al. 1992), this parasitoid showed a good ability to establish in that area and a promising potential to control DBM populations. High temperature however, limited the effectiveness of *D. semiclausum*. Application of Bt was suggested for complementary control, especially under such conditions. In Georgia, under severe DBM pressure in Summer, more frequent application of Bt is needed (Chalfant 1992).

In these experiments, residual activity of Bt and the effect of various temperatures on mortality of DBM larvae treated with Bt were examined.

### Materials and Methods

**Residual Effect of Bt.** The experiment was carried out in the insectary ( $27 \pm 1$  °C, average RH  $27.1 \pm 7.2$  %, and approximately 8 hours light per day) using the leaf-dip method. Concentrations used were 153, 334, and 2237 International Units of *B. thuringiensis* potency per ml plus control. Ten larvae per treatment were placed on the treated leaves after 2, 48, 96, 144, and 192 hours.

Five replications were performed for this experiment. Mortality was recorded daily. Mortality data were corrected against mortality of control using Abbott's formula (Abbott 1925). Two-way ANOVA was used to analyze the effect of time, dosages, and their interaction. Probit analysis was used to obtain LC<sub>50</sub>s from the treatments (Finney 1971).

**Temperature Effect on Bt.** A similar method as in the residual testing was used in this experiment. Concentrations were the same as above. Larvae were placed on the leaves 2 hours after the leaves were treated. Growth chambers were used for the temperatures tested, which were 15, 20, and 30 °C. There were five replications for each temperature tested. Data obtained were analyzed the same way as in the residual testing.

## Results and Discussion

**Residual Effect of Bt.** Results of two-way ANOVA of this experiment showed no interaction between concentrations and residual activity ( $P > 0.10$ ,  $F$  value = 0.91,  $df = 8$ ). Exposing DBM larvae to leaves after being treated 2, 48, 96, 144, and 192 hours did not cause any significant difference in larval mortality ( $P = 0.6691$ ,  $F$  value = 0.59,  $df = 4$ ) (Fig. 3). This indicates that under room conditions, where direct solar radiation was eliminated, Bt retains its potency up to 8 days. Differences, however, were obtained from the various dosages as higher concentrations resulted in highly significant differences in mortality ( $P < 0.001$ ,  $F$  value = 166.3,  $df = 4$ ). The variation in mortality between concentrations explained 83 % of the total variation, while that between time differences explained only 0.6 %. Fifteen percent of the total variation were not explained by the model.  $LC_{50}$ s obtained from Probit Analysis are presented in Table 6. and Fig. 4. Means from treatments are summarized in Table 7.

In contrast, under wet field conditions, residual effect of Bt for control of defoliating forest insects was low. van Frankenhuyen and Nystrom (1989) observed that, under rainy conditions, residual toxicity half life of a high protein Bt formulation was 1 - 2 days. A high concentration did not improve foliar persistence. A suitable sticker was required. The experiment also showed that the mean mortality 0 day after treatment was significantly different from all other treatments. Loss of residual toxicity of 50 - 90 % occurred in the treatment where foliage was exposed to sun and rain, but when the foliage was sheltered from sun and rain, only 15 % toxicity loss occurred.

The field experiment by Beckwith and Stelzer (1987) did not show as rapid degradation as indicated by van Frankenhuyen and Nystrom (1989). The data

demonstrated significant negative linear regression of persistence with time. At day 10, however, mortality was 13 to 40 % of the original.

My results indicated that potency of Bt toxins is not decreased after being applied up to eight days under room conditions, while in the experiment by van Frankenhuyen and Nystrom (1989) sheltering foliage from sun and rain only retained residual toxicity up to 4 days. Therefore, the short residual activity of Bt in the field is more likely to be due to extrinsic factors rather than the intrinsic characteristics of Bt. These data suggest that the effectiveness of Bt in the field can be conserved by improving the formulation product.

Efforts have been made to enhance the performance of Bt in the field. MVP is a formulation of Bt products in which a selected delta endotoxin of Bt subsp. *kurstaki*, which is highly toxic to DBM, is encapsulated and stabilized within dead bacterial cells to protect the toxin from environmental degradation. Field evaluation demonstrated that this form of Bt product provides residual activity 2 to 3 times greater than Dipel 4 L and Javelin which contain unprotected toxin crystals. Laboratory tests evaluating the effect of UV radiation on the efficacy of Bt products indicated that the persistence of MVP was 5 - 36 times greater than those of Dipel, Torrow-CT, and Bacilex (Soares and Quick 1992).

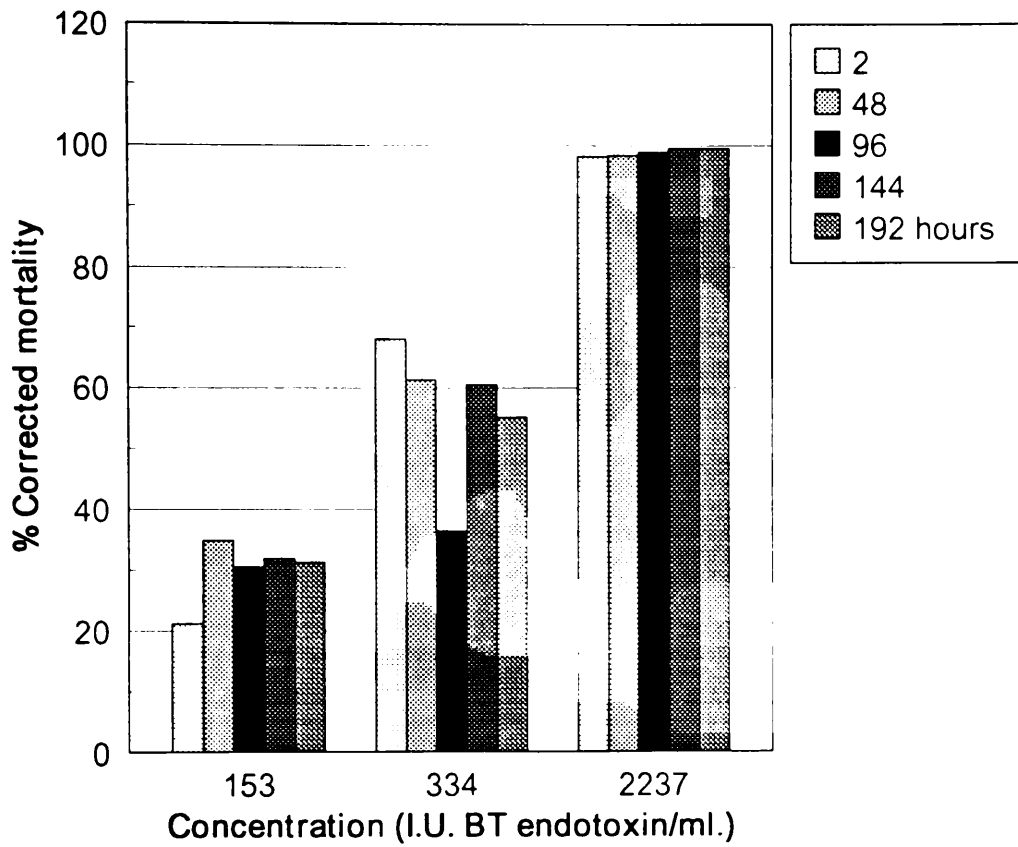


Fig. 3. Residual effect of Bt on mean mortality of DBM larvae

Table 6. Response of third instar DBM to leaf residue of Bt under laboratory conditions.

Hours after treatment	n <sup>a</sup>	LC <sub>50</sub> <sup>b</sup>	Intercept	Slope ± Std error	95 % Conf. interval
2	216	298	-0.32	2.150 ± 0.332	59.7 - 1483.9
48	211	247	0.58	1.849 ± 0.311	35.6 - 1709.9
96	256	359	-0.84	2.286 ± 0.288	88.4 - 3588,3
144	204	248	-9.16	2.127 ± 0.367	44.9 - 1369.4
192	224	175	0.25	2.151 ± 0.337	57.2 - 1393.7

<sup>a</sup> total larvae

<sup>b</sup> International Units of *B. thuringiensis* potency per ml.

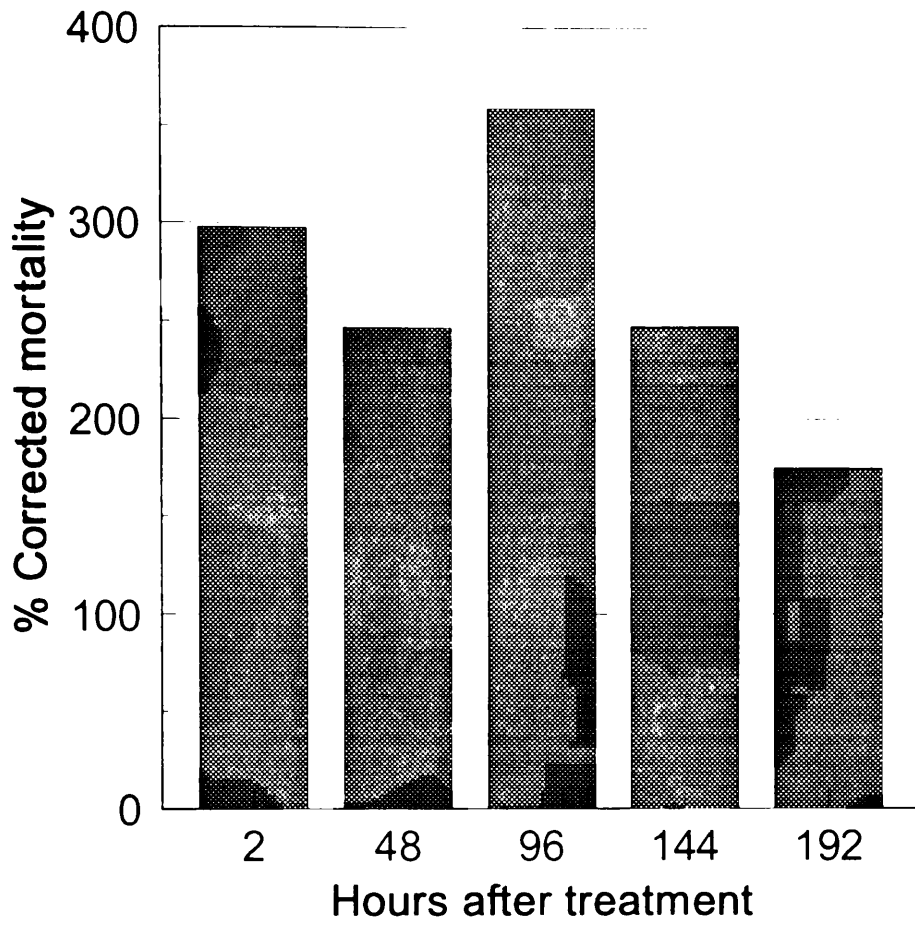


Fig.4. Residual effect of Bt on LC<sub>50</sub> of third instar DBM

Table 7. Residual effect of BT on third instar DBM <sup>a</sup>.

Treatment Concentration <sup>c</sup> /hours		Mean $\pm$ Std Error <sup>b</sup>
153	- 2	21.9 $\pm$ 6.4 a
153	- 48	34.9 $\pm$ 4.7 ab
153	- 96	30.6 $\pm$ 9.8 ab
153	- 144	31.9 $\pm$ 7.9 ab
153	- 192	31.3 $\pm$ 3.5 ab
334	- 2	68.1 $\pm$ 13.7 bcd
334	- 48	61.2 $\pm$ 4.3 bcd
334	- 96	36.5 $\pm$ 5.4 abc
334	- 144	60.5 $\pm$ 7.9 bcd
334	- 192	55.2 $\pm$ 3.7 bcd
2237	- 2	98.2 $\pm$ 2.8 e
2237	- 48	98.4 $\pm$ 2.4 e
2237	- 96	99.0 $\pm$ 4.9 e
2237	- 144	99.6 $\pm$ 1.8 e
2237	- 192	99.6 $\pm$ 2.0 e

<sup>a</sup> Five replications

<sup>b</sup> Means (corrected % mortality ) followed by the same letter are not significantly different at the 0.05 level

<sup>c</sup> International Units of Bt potency per ml.

**Temperature Effect on Bt.** In this study, increasing temperature from 15 °C to 20 °C increased the LC<sub>50</sub> from 532 to 659 International Units (I.U.) of Bt. The LC<sub>50</sub> decreased, however, to 219 I.U. at 30 °C. Two-way ANOVA indicated that mortality at 30 °C was significantly different from that at 15 and 20 °C (Table 8). No significant difference was found between mortality at 15 and 20 °C. There was no significant interaction between temperature and concentration. The main effect of concentration was highly significant ( $P < 0.001$ ,  $F$  value = 42.3,  $df = 2$ ) and explained 75% of the total variation in the mortality. The main effect of temperature was also significant ( $P = 0.02$ ,  $F$  value = 4.13,  $df = 2$ ), but the variation between temperatures explained only 6 % of the total variation. Twenty nine percent of the total variation was not explained by the model. Mean mortality of treatments are presented in Table 9 and Fig. 5.

Mean time of mortality decreased with increased temperature, being  $11.2 \pm 0.9$ ,  $6.0 \pm 0.5$ , and  $3.8 \pm 0.6$  days, at 15 °C, 20 °C and 30 °C respectively (Table 10). Mean time of mortality at 20 °C and 30 °C are not different significantly, but are significantly lower than that at 15 °C ( $P = 0.0001$ ,  $F$  value = 29.42,  $df = 2$ ). van Frankenhuyen (1990) indicated that temperature, ranged from 13 - 25 °C, significantly affected the progression of mortality but not the final level of cumulative mortality. He reported that LT<sub>50</sub> decreased from 12 - 17 days at 13 °C to 2 - 4 days at 25 °C.

From these results, it is apparent that DBM larvae were more sensitive to Bt at 30 °C than the lower temperatures tested. It is possible that this temperature weakened the larvae (Wakisaka et al. 1992) and made them more susceptible to Bt, which is still effective at this temperature (Kissinger and McGaughey 1976). These phenomena beneficially contributed to the compatibility of Bt with other tactics (control agents) which are less effective at high temperatures.

Studying the effects of storage temperatures on the persistence of Bt during extended storage, Kissinger and McGaughey (1976) reported that temperature as high as 38 °C on 39 days during summer did not cause deterioration in toxicity. Insecticidal activity did not decrease appreciably at storage temperatures of 16.5, 25 or 33.5 °C but decreased at 42.0 °C after storage of approximately 15 weeks. Results of studies by Wakisaka et al. (1992) showed that temperature higher than 30 °C delayed the development and reduced the survival of immature stages of DBM.

Table 8. Effects of temperature on mean mortality of third instar DBM and LC<sub>50</sub> of Bt.

Temp. °C	n <sup>a</sup>	Mean±SE <sup>b</sup>	LC <sub>50</sub> <sup>c</sup>	Slope + Std error	95 % Conf. interval
15	211	54.7 ± 7.5a	532.2	1.581 ± 0.234	67.4 - 4204.5
20	266	56.3 ± 7.5a	659.4	1.470 ± 0.202	90.4 - 4812.0
30	227	75.0 ± 7.5 b	219.4	1.879 ± 0.320	34.4 - 1400.0

<sup>a</sup> total larvae.

<sup>b</sup> Means followed by the same letter are not significantly different at the 0.05 level.

<sup>c</sup> International Units of *B. thuringiensis* potency per ml.

Table 9. Mean mortality of third instar DBM treated with Bt at different temperatures<sup>a</sup>.

Concentration <sup>b</sup> /Temp.(°C)	Mean ± Std. error <sup>c</sup>
152 - 15	22.0 ± 6.2 a
152 - 20	40.6 ± 6.7 a
152 - 30	42.7 ± 11.0 ab
334 - 15	48.2 ± 11.8 ab
334 - 20	42.8 ± 13.4 ab
334 - 30	60.3 ± 7.4 b
2237 - 15	88.0 ± 4.7 c
2237 - 20	90.6 ± 8.3 c
2237 - 30	99.3 ± 3.3 c

<sup>a</sup> Five replications

<sup>b</sup> International Units of Bt potency per ml

<sup>c</sup> Means (corrected percent mortality) followed by the same letter are not significantly different at the 0.05 level.

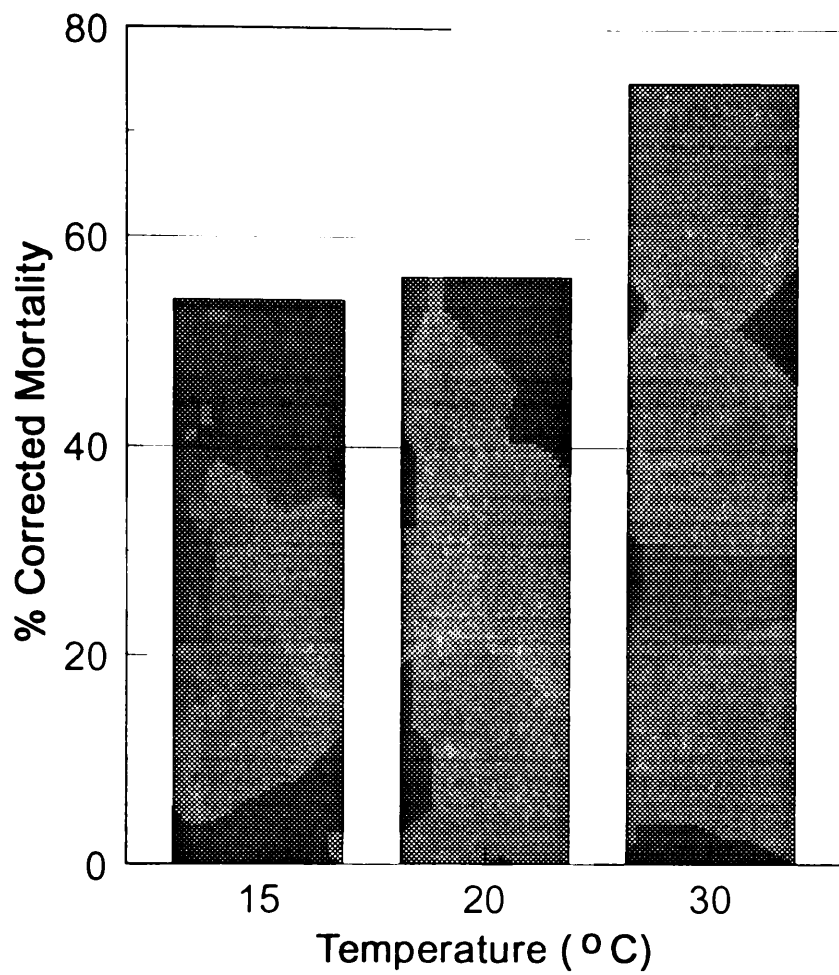


Fig. 5. Effect of temperature on mean mortality of third instar DBM subjected to Bt

Table 10. Mean time of mortality of third instar DBM subjected to different concentrations of Bt at different temperatures.

Concentration <sup>a</sup>	Mean time (Days)		
	15°C	20 °C	30 °C
Control	9.6	5.2	4.5
153	13.7	5.1	5.2
334	11.1	7.1	3.1
2237	10.2	6.5	2.5
(Mean ± SE) <sup>b</sup>	(11.2 ± 0.9 a)	(6.0 ± 0.5 b)	(3.8 ± 0.6 b)

<sup>a</sup> International Units of Bt potency per ml.

<sup>b</sup> Means for each temperature followed by the same letter are not significantly different at the 0.05 level.

## Summary

The susceptibility of a population of diamondback moth (DBM), *Plutella xylostella* L. (Lepidoptera: Plutellidae), collected from two locations in Montgomery County, Virginia, and its ability to develop resistance to *Bacillus thuringiensis* was evaluated. The field population of DBM in this area was found to be susceptible to *Bacillus thuringiensis* (Bt). Selection pressure for nine generations with Bt at a concentration of 153 I.U./ml did not cause any significant difference in mean mortality of third instar DBM. However, there was a trend towards lower mortality with significant negative linear correlation ( $P = 0.003$ ,  $r^2 = 0.73$ ). Since crucifer crops in Virginia are not cultivated continuously, therefore the selection pressure of Bt on DBM is low and development of Bt resistance in DBM in this area might not occur in the near future. However, since DBM has the ability to migrate over long distances and may carry resistance genes with them, it is appropriate to be alert to the occurrence of Bt resistance.

The interrelationship of *B. thuringiensis* to diamondback moth and its primary parasitoid, *Diadegma insulare* Cress. (Hymenoptera: Ichneumonidae), was studied by determining: the differential response of third instar diamondback moth, parasitized and unparasitized, to *B. thuringiensis*; and the ability of *D. insulare* to discriminate between *B. thuringiensis*-treated and untreated hosts. Lower mortality was observed from parasitized DBM larvae when compared with that of unparasitized larvae at each of the three concentrations consisting of 153, 334, 2237 I.U of Bt endotoxin per ml. The differences between the mean mortality of parasitized and unparasitized larvae at each of the three treatment levels are not significant ( $P > 0.05$ ). The regression lines of the response of parasitized and unparasitized larvae to Bt, however, are highly significant ( $P = 0.0001$ ). These data, and results of the study by McDonald (1990) and Idris and Grafius (1993b)

suggest the compatibility of Bt and natural enemies in the field. Female *D. insulare* did not show the ability to discriminate between *B. thuringiensis*-treated and untreated hosts. This indicates that Bt may induce indirect mortality to *D. insulare*. The percentage of *D. insulare* females emerging from Bt-treated larvae (41.4 %) was not different significantly from that of untreated larvae (32.0 %).

Residual activity of Bt under room conditions and the effect of various temperatures on mortality of DBM larvae treated with Bt were examined. The effects of leaf residue of *B. thuringiensis* endotoxin were not significantly different between 2 and 192 hours after treatment. This indicates that under room conditions, Bt retains its potency up to 8 days. The short residual activity of Bt in the field (van Frankenhuyen and Nystrom 1989), therefore, is more likely to be due to extrinsic factors rather than intrinsic characteristics of Bt. Mean mortality of third instar diamondback moth subjected to *B. thuringiensis* endotoxin at 153, 334, and 2237 I U/ml were not significantly different at temperatures of 15 and 20 °C, but were significantly lower than that at 30 °C. The mean time of mortality decreased with increasing temperature. The means are  $11.2 \pm 0.9$  days,  $6.0 \pm 0.5$  days, and  $3.8 \pm 0.6$  days, respectively at 15, 20, and 30 °C. These results show that temperature affects the progression of larval mortality and susceptibility of third instar DBM at 30 °C. These data suggest a complementary action of Bt to the role of parasitoids since parasitization by *D. eucerothaga* was reported (Talekar and Yang 1991) to be reduced sharply at temperatures approaching 30 °C.

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## VITA

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