Interrelationship of *Bacillus thuringiensis* Berliner to the Diamondback Moth (Lepidoptera: Noctuidae) and its Primary Parasitoid, *Diadegma insulare* (Hymenoptera: Ichneumonidae)[1]

S. Ulpah[2] and L. T. Kok

Department of Entomology
Virginia Polytechnic Institute and State University
Blacksburg, VA 24060-0319 U.S.A.

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ABSTRACT The interrelationship of *Bacillus thuringiensis* Berliner var. *kurstaki* to the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), and its primary parasitoid, *Diadegma insulare* Cress. (Hymenoptera: Ichneumonidae), was investigated using laboratory colonies of the insects. The differential response of third-instar diamondback moth, parasitized and unparasitized, to *B. thuringiensis* (*Bt*), and the ability of *D. insulare* to oviposit in B*t*-stressed hosts were determined. No significant difference ($P > 0.05$) was found between the mean mortality of parasitized and unparasitized larvae at each of three concentrations (154, 334, and 2,237 IU/ml) of *Bt* endotoxin. The three concentrations were equivalent to the LC_{30}, LC_{50}, and LC_{90} of *Bt* potency based on preliminary tests. Parallel line assay analysis, however, revealed that the linear dose-response regressions of parasitized and unparasitized larvae were highly significant ($P = 0.0001$). The LC_{50}s of parasitized versus unparasitized larvae were 373 and 175 IU/ml *Bt* endotoxin, respectively, indicating that parasitized larvae were less susceptible to *Bt*. Female *D. insulare* oviposited in *Bt*-stressed hosts. The percentage of *D. insulare* females emerging from *Bt*-treated larvae (41.4%) was not significantly different from that of untreated larvae (32.0%).

KEY WORDS *Plutella xylostella*, Plutellidae, diamondback moth, *Diadegma insulare*, parasitoid, Ichneumonidae, Hymenoptera, *Bacillus thuringiensis*

The use of *Bacillus thuringiensis* Berliner (*Bt*) as a 'safe' insecticide to non-target organisms has generated much interest on responses of predators and parasitoids to *Bt*. Available data show a range of responses, depending on parasitoid species and dosage of *Bt*. Some evidence suggests that *Bt* may induce mortality in beneficial non-target arthropods, although it is not clear whether the mortality is directly induced by the *Bt* toxin or indirectly caused by the deteriorating quality of the host of the parasitoid larvae. Salama et al. (1982) reported that *Microplitis demolitor* Wlkr. [a parasitoid of cotton leafworm,

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[2] Current address: Faculty of Agriculture, Islamic Univ. North Sumatra, Jl. S. M. Raja No. 191, Medan 20217, Indonesia.

Spodoptera littoralis (Boisd.)] fed on a diet containing *Bt* showed significant reduction in percentage of emergence and reproductive potential. Hamed (1979) reported that five parasitoid species of *Yponomeuta evonymellus* (Lepidoptera: Yponomeutidae) were sensitive to *Bt* if they ingested spores of *Bt* with food. McDonald et al. (1990) showed that *Cotesia rubecula* (Marshall) (Hymenoptera: Braconidae) cannot successfully emerge after exposure to one-tenth of the recommended field dosage of *Bt*, but emergence was not affected by lower dosages.

Conversely, some studies showed a synergistic effect of *Bt* on parasitoids. Weseloh et al. (1983) reported that percent parasitization of the gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae), by a braconid was higher in plots treated with *Bt* than in untreated plots. The retarding effect of larval development caused by *Bt* on the gypsy moth provided the braconid with a large number of caterpillars. Similar results were obtained by Wallner et al. (1983).

Other reports suggest no deleterious effects of *Bt* on hymenopterous parasitoids. For example, McDonald et al. (1990) showed that fourth-instar *Pieris rapae* L. parasitized by *C. rubecula* were less susceptible to low dosages of *Bt* than unparasitized larvae. Studies by Muck et al. (1981) on *Apanteles glomeratus* L. (Hymenoptera: Braconidae) and *Pimpla turionella* L. (Hymenoptera: Ichneumonidae) 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 *D. insulare* adults even at high concentrations.

The exact amount of *Bt* consumed by larval parasitoids is difficult to quantify, but if consumption of *Bt* makes parasitized hosts feed less (Flexner et al. 1986), then the parasitized hosts' exposure to *Bt* is reduced and their mortality is decreased. 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 be no 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 *D. insulare* to successfully develop in larvae that survived *Bt* treatment (*Bt*-stressed) would increase their compatibility with *Bt*. Also, proper timing of *Bt* application and parasitoid release, e.g., releasing *D. insulare* before *Bt* application, might enhance the compatibility of *Bt* and the parasitoid if there was a differential response between parasitized and unparasitized diamondback moth larvae to *Bt*. Thus, the objectives of this study were to determine if there is a differential response to *Bt* by parasitized and unparasitized diamondback moth larvae, and whether the parasitoid, *D. insulare*, would oviposit in *Bt*-stressed larvae.

Materials and Methods

Colonies of *D. insulare* and diamondback moth reared on cabbage were maintained at the insectary of VPI & SU, Blacksburg. The microbial insecticide used was *Bt* subsp. *kurstaki* (Dipel® 2X, Abbot Laboratories, North Chicago, IL; 32,000 IU/mg) with recommended rate of 0.90 g/l for cabbage. We used *Bt* at concentrations equivalent to LC_{30}, LC_{50}, and LC_{90} (153, 334, and 2,237 IU/ml of *Bt*, respectively) obtained from a preliminary study plus a control (Ulpah 1994).

Bioassays were conducted using a leaf-dip method. Two young cabbage leaves (8.0 cm width) were dipped in each concentration of *Bt* for 30 seconds and left to dry for about 2 h. For control, leaves were dipped in water. The petioles of the leaves were inserted through a hole of an inverted plastic container lid (12 cm diam) into 200 ml of water in a jar. A ventilated plastic container of 0.9 liter was used to cover the leaves after larvae were placed on them. The tests were conducted at 26.7 ± 1.0°C.

Differential response of diamondback moth, parasitized and unparasitized by *D. insulare*, to *Bt*. To obtain parasitized and unparasitized larvae at approximately the same age, four potted cabbage plants were placed in each oviposition cage with about 50 diamondback moths. There were six of these oviposition cages. Cabbage plants were changed every week. Half of the potted cabbage with diamondback moth third instars was placed in "sting" cages with 30 to 40 *D. insulare* females and another half was kept in cages without the parasitoids. The plants in the sting cages were removed from the cages after 24 h to obtain parasitized larvae for the experiment. Ten diamondback moth third instars were used per replication; there were six replications for both parasitized and unparasitized larvae. Treatments 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). LC_{50}s for parasitized and unparasitized larvae subjected to the selected dosages of *Bt* were obtained from probit analysis (SAS Proc Probit). Data were analyzed using Proc GLM (SAS Institute 1990), and Tukey's studentized range (HSD) test was used to determine significance of difference between treatments. Linear dose response regressions of *Bt* for parasitized and unparasitized larvae also were compared by Parallel Line Assays Analysis (Finney 1978).

Ability of *D. insulare* to oviposit in *Bt*-stressed larvae. Early diamondback moth third instars were fed cabbage leaves treated with *Bt* at a concentration equivalent to LC_{90} (2,237 IU/ml) for 24 h. For control, the same age larvae were fed untreated leaves. The treated larvae and control were exposed together to *D. insulare*.

About 10 larvae were used per unit experiment, replicated 10 times. We used a marker to place a dot on the back (dorsoabdomen) of the larvae to differentiate between treated and untreated larvae. To investigate the marking effect, half of the treated larvae was 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. A female parasitoid was placed in each experimental unit for about 3 h before treated larvae were separated from the control. We recorded mortality and adult emergence daily and calculated percent parasitization based on the emerging adults.

Student's *t*-test was used to determine whether there was any difference in the oviposition of *D. insulare* in *Bt*-stressed and untreated hosts. The same analysis was used to determine artificial marking effects on parasitism. The proportions of female parasitoids emerging from treated and untreated larvae were compared using *z*-test analysis (Zar 1984).

Results and Discussion

Differential response of diamondback moth, parasitized and unparasitized by *D. insulare*, to *Bt*. Differences in mortality of third-instar diamondback moth due to the different dosages of *Bt* treatment were highly significant ($F = 12.07$; df = 7; $P = 0.0001$). Mortality of parasitized larvae was generally lower than that of unparasitized larvae (Table 1), although differences between mortality of parasitized and unparasitized larvae at each of the three treatment levels were not significant (HSD = 43.2, n = 400, $\alpha = 0.05$). Parallel Line Assays Analysis, however, showed that the linear dose-response regression of *Bt* for parasitized and unparasitized larvae was highly significant ($F = 27.8$; df = 1; $P = 0.0001$). LC_{50}s of parasitized and unparasitized larvae obtained from Probit Analysis were 372.7 (95% CL = 290.8 - 430.6) and 174.5 (95% CL = 26.1 - 271.8) IU/ml, respectively. The higher LC_{50} value for the parasitized larvae indicated that parasitized larvae were less susceptible to *Bt* than unparasitized larvae. Similar results were obtained by McDonald et al. (1990) where *P. rapae* larvae parasitized by *C. rubecula* were less susceptible to low dosages of *Bt* than unparasitized larvae.

Idris and Grafius (1993b) reported different results of a similar experiment. They found no significant difference between mortality of parasitized and unparasitized larvae of diamondback moth in the *Bt* treatment. They attributed the lack of difference in mortality to the same amount of food being consumed by parasitized and unparasitized larvae. They also observed that a greater proportion of adult parasitoids from larvae surviving pesticide treatments tended to be females than from the control group.

Table 1. Mortality of parasitized and unparasitized diamondback moth larvae fed cabbage leaves dipped in three different concentrations of *Bt*.

Concentration (IU/ml)	% Corrected mortality (Mean ± SE)*	
	Parasitized	Unparasitized
0 (Control)	0	0
153	30.2 ± 12.2c, x	38.1 ± 5.5b, x
334	45.1 ± 13.4bc, y	61.6 ± 5.9ab, y
2237	80.0 ± 7.8ab,z	87.5 ± 4.7a, z

*Means of 6 replicates (n = 55 to 79). Means with the same letter in each row (x,y,z) or column (a,b,c) are not significantly different [$P > 0.05$, Tukey's test (Zar 1984)].

Because parasitized larvae showed 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), immature parasitoids were probably able to complete their development before the hosts consumed a lethal amount of *Bt*. This interaction suggests high compatibility of *D. insulare* and *Bt* in the field because larvae that escape from parasitism are expected to acquire a lethal amount of *Bt* toxins as they continued feeding. Flexner et al. (1986) stated that when *D. insulare* release is combined together with use of *Bt* for integrated pest management, application of *Bt* after parasitoids have infested the hosts would greatly reduce indirect mortality of the beneficial insects.

Ability of *D. insulare* to oviposit and develop in Bt-stressed larvae. In this study, percent parasitization was based on the total number of emerging adults (*D. insulare* and diamondback moth). Parasitism of non-surviving larvae (larvae died because of *Bt* treatment) was not included. Analysis of variance from the untreated and *Bt*-stressed larvae showed no significant difference (F = 0.23; df =1, 3; P = 0.874). This indicated that the parasitoid was able to oviposit and successfully develop in *Bt*-stressed larvae. Student's t-test analysis of pooled data (marked and unmarked) between *Bt*-treated and untreated larvae revealed no significant difference (t = 2.649E-04; df = 38; P = 0.454). To evaluate marking effects on parasitism, data were pooled from *Bt*-treated and untreated treatments for t-test analysis; no significant difference was found (t = 0.432; df = 38; P = 0.586).

These results indicated that *D. insulare* did oviposit in *Bt*-stressed diamondback larvae. However, observations were on *Bt*-stressed larvae that survived the treatment. It is not clear whether the parasitism of *Bt*-stressed larvae was due to the inability 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 in *Bt*-treated larvae that will eventually die and not 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 (193), 68.9% were *D. insulare*. Of emerging adult parasitoids, 36.1% were female. There was no significant difference in the proportion of female parasitoids emerging from *Bt*-stressed (41.4%) and non-treated (32.0%) larvae (z value = 1.1168, P = 0.2640). If we assume that *Bt* treatment killed diamondback moth larvae containing the same proportion of female and male parasitoids, these data are not in agreement with either the results of Idris and Grafius's (1993b) where more *D. insulare* females were obtained from diamondback moth larvae surviving *Bt* and other insecticide treatments; or the results of Wallner et al. (1983), where reduced size of *Lymantria dispar* L. larvae treated with *Bt* caused *Rogas lymantriae* Watanabe (Hymenoptera: Braconidae) to oviposit more unfertilized eggs, resulting in 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 which have been subjected to *Bt* would give valuable information on the resulting population dynamics of *D. insulare*.

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