Effect of Beta·Exotoxin of *Bacillus Thuringiensis* on Development of the Mexican Bean Beetle (Coleoptera: Coccinellidae)

George E. Cantwell
USDA Agricultural Research Service

William W. Cantelo
USDA Agricultural Research Service

Michael A. Cantwell
Maryland Department of Agriculture

EFFECT OF BETA-EXOTOXIN OF *BACILLUS THURINGIENSIS* ON DEVELOPMENT OF THE MEXICAN BEAN BEETLE (COLEOPTERA:COCCINELLIDAE)

George E. Cantwell[1], William W. Cantelo[1], and Michael A. Cantwell[2]

ABSTRACT

Mexican bean beetle larvae were fed several levels of *Bacillus thuringiensis* var. *thuringiensis* that contained the beta-exotoxin to determine if this treatment would extend or prolong their preadult life span. Feeding on bean foliage sprayed with a solution containing 2 g/liter active ingredient diluted 1:10,000, extended the life by 7.5 days. Dilutions of 1:15,000 and 1:20,000 extended the life by over 3 and 5 days, respectively. By extending the time of larval development, enhancement of parasite or predator activity may occur.

It has been established that the beta-exotoxin of *Bacillus thuringiensis* var. *thuringiensis* (*B.t.t.*) will kill the Mexican bean beetle, *Epilachna varivestis* Mulsant, (MBB) larvae (Cantwell and Cantelo 1982), and that this material has no apparent effect on certain predators and parasites of this and other beetles. One of the larval parasites of the MBB, *Pediobius foveolatus* Crawford (Hymenoptera:Eulophidae), which is also effective in reducing field populations of the MBB, has been used in conjunction with *B.t.t.* beta-exotoxin by Cantwell et al. (1985), the combination producing an additive killing effect which resulted in excellent control of this pest.

Other researchers have shown that sublethal doses of *B.t.t.* will enhance the activity of parasites of other insect species by extending the time of larval development, thereby allowing a longer time period in which the parasite may operate. Ticehurst et al. (1982) reported a one-week temporal lag in development of gypsy moth larvae when fed Dipel, a commercial product of *B.t.* which does not contain the beta-exotoxin.

The purpose of this study was to determine if the *B.t.t.* exotoxin produced such a lag in larval development of the Mexican bean beetle.

MATERIALS AND METHODS

Laboratory test. An experimental formulation of *B.t.t.* tested in this experiment was supplied by Sandoz Inc., and referred to as SAN410SC-72. Dilutions of this material, which contained 2g/liter of the beta-exotoxin, were 1:5000, 1:10,000, 1:15,000, and 1:20,000. The test dilutions of *B.t.t.* were sprayed on upper and lower leaf surfaces until run-off on greenhouse-produced lima bean (*Phaseolus lunatus* L.) foliage (cultivar 'Henderson'). Controls were sprayed with water. After drying, the primary leaves were removed and placed singly on discs of moistened filter paper in large petri dishes (125 by 25 mm). Neonate MBB larvae, one per petri dish, were transferred onto the leaf. All dishes

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[1]Vegetable Laboratory, Agricultural Research Service, USDA, BARC-East, Beltsville, MD 20705.

[2]Plant Protection Section, Maryland Department of Agriculture, Annapolis, MD 21401.

were held at 20°C and 65% RH with 15:9 L:D regime. Fresh, treated foliage was added as needed at least once every 4 days during instars 1 through 3, and at least once every other day through instar 4. Each replication consisted of between 10 to 30 dishes. Observations were made daily and the stage of each individual was noted.

All dishes were maintained until adult eclosion or death of the individual. Treatment groups comparing the various levels of SAN410SC-72 were as follows: Control, five replications with 90 individuals total; 1:5000, two replications with 40 individuals total; 1:10,000, three replications with 60 individuals total; 1:15,000, three replications with 60 individuals total; and 1:20,000, four replications with 80 individuals total.

The number of days per stage and the total development time of the MBB larvae were calculated. The variances were analyzed to determine if significant differences existed in the development time of each stage. Differences between the means were determined by Duncan's multiple range test.

Field test. In 1984 a test was conducted at Beltsville, Maryland, using *B.t.t.* at dilutions of 1:500 and 1:1000 on field-grown beans. Snap beans (*Phaseolus vulgare* L.), 'Provider' cultivar, were planted in rows 45 cm apart on 17 May at a depth of 25–30 mm. After germination a field cage measuring 2 by 2 by 2m was placed over four adjoining rows of beans at every 4 m along the row for a total of 21 cages. On 8 June the cages were separated into three treatment-level groups and the beans therein sprayed. Spray was applied with compressed air sprayers. Controls were sprayed with water. After drying, each cage was infested with 100 2nd instar, 96-h-old MBB larvae. Spray treatments were repeated at weekly intervals for 4 weeks.

Weekly sampling commenced on 15 June and continued through 29 June. Sampling procedure consisted of randomly selecting three different sections of plant rows 0.7 m in length per cage, and recording the number and stage of individual MBB encountered. Final data were collected on 6 July by recording all MBB in each cage.

RESULTS AND DISCUSSION

Feeding on foliage treated with sublethal doses of *B.t.t.* beta-exotoxin increased the longevity of the MBB as the data in Table 1 indicate. Although feeding on the highest concentration of 1:5000 resulted in 100% mortality before the 3rd instar, feeding on the next lower concentration of 1:10,000 extended the preadult time by nearly 7.5 days. This represents a 40% increased beetle development time during which parasitism or predation may take place. Feeding on more dilute concentrations 1:15,000 and 1:20,000 extended the preadult life by over three and five days respectively. At the highest sublethal dose tested, each of the life stages except that of the prepupa was extended significantly past that of the untreated. All of the treatment levels significantly extended the insects time in the 1st and 2nd instar. Extension of preadult life stages of the MBB may improve the likelihood of parasitism by *Pediobius foveolatus* since its host range is the 2nd instar through the prepupal stages of the Epilachnini.

This extension of the preadult life span is similar to the situation described by Retnakaran and Bird (1972) in poxvirus-infected spruce budworm, *Choristoneura fumiferana* (Clemens), (Lepidoptera:Tortricidae) and also by Doughterty et. al. (in press) in granulosis-infected cabbage loopers, *Trichoplusia ni* (Hübner), (Lepidoptera:Noctuidae). It has also been reported that some microorganisms such as the protozoan parasite of *Tribolium confusum* Jacquelin DuVal produce a substance that mimics juvenile hormone and its effects are manifested by time extensions in preadult development (Fisher and Sanborn 1964).

It may be speculated that the *B.t.t.* exotoxin has a directly or indirectly stimulating effect on the beetle's corpora allata thereby increasing the release of enough juvenile hormone to maintain the larval state. Such false juvenilizing effects have also been produced by other compounds such as colchicine, oleic acid, methanol, and certain carbamate insecticides, (Hintze-Podufal 1971, Slama 1961, Smith 1971). However, since

Table 1. Effect of the beta-exotoxin of *Bacillus thuringiensis* var. *thuringiensis* on longevity of the Mexican bean beetle

Treatment dilution[b]	Mean number of days in each life stage[a]					Total time 2nd-pupa
	1st and 2nd Instar	3nd Instar	4rd Instar	Prepupa	Pupa	
Control	3.85B	4.16B	3.82D	4.15A	2.80B	18.8
1:20,000	5.58A	3.39C	4.69C	4.46A	3.90AB	22.0
1:15.000	5.27A	4.16B	5.45B	4.94A	4.06A	23.9
1:10,000	5.00A	5.17A	7.25A	4.57A	4.29A	26.2
1:5000	5.71A	dead	—	—	—	—

[a]Means followed by the same letters in the same column are not significantly different at the 5% level using Duncan's multiple range test.
[b]SAN410SC-72 (2g/1 A.I.) supplied by Zoecon Corp., Wasco, California.

the treatments did not produce extra larval instars but an extended time in each instar, a toxic suppression of feeding or metabolism may be as likely an explanation as stimulating the release of juvenile hormone.

Another result of the upsetting or disruption of the delicate hormonal balance may be the production of teratological conditions in insects. In the Coleoptera, feeding by larvae of the Colorado potato beetle on sublethal doses of *B.t.t.* beta-exotoxin not only extended the larval life-span but was responsible for teratologies in 6% of the adults as reported by Burgerjon and Biache (1967) and Burgerjon et. al. (1969), and later confirmed by Cantwell and Cantelo (1981).

Because of the high concentrations or frequency of *B.t.t.* applications used in the field test, most of the 100 larvae released into each cage did not survive to the pupal stage, hence we were unable to determine if feeding on the beta-exotoxin caused a time lag in beetle development in the field. At the dilution of 1:500 and 1:1000 only 11 of the 1400 beetles reached the pupal stage, and only three became adults, compared to the untreated where 482 adults formed from the 700 larvae released.

Our work indicates that feeding on sublethal doses by preadult stages of the Mexican bean beetle on *Bacillus thuringiensis* containing the beta-exotoxin does indeed extend the time spent in the various larval stages. This extended time provides a longer time period in which parasites and predators may operate in further reducing the beetle population. Additional work is needed to determine if this added benefit of extended time is offset by feeding damage that may occur during this period.

Table 2. Effect of *Bacillus thuringiensis* exotoxin on development of the Mexican bean beetle.

Treatment dilution	Total Number of Mexican Bean Beetles on Each Sampling Date[a]											
	15 June			22 June			29 July			6 July		
	L	P	A	L	P	A	L	P	A	L	P	A
Control	183	0	0	44	130	0	0	33	263	0	0	482
1:1000	33	0	0	15	5	0	0	5	2	0	0	3
1:500	28	0	0	18	1	0	0	6	0	0	0	0

[a]Total number of Mexican bean beetles on 14-m row of caged beans per treatment (2-m row per each of seven cages per treatment). L = larvae, P = pupae, A = adults.

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