THE INTEGRATION OF A BACTERIUM AND PARASITES TO CONTROL THE COLORADO POTATO BEETLE AND THE MEXICAN BEAN BEETLE

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ABSTRACT

Sprays of *Bacillus thuringiensis* subsp. *thuringiensis (B.t.t.),* which contained the beta-exotoxin, were applied to small-scale field plots of tomatoes infested with the Colorado potato beetle *[Leptinotarsa decemlineata* (Say)] and to bean plots infested with the Mexican bean beetle *(Epilachna varivestis* Mulsant). Two parasitic wasps of the family Eulophidae were used in conjunction with the *B.t.t.* in an effort to reduce beetle populations. Treatments on tomatoes consisted of either *B.t.t.* alone, *B.t.t.* plus releases of the egg parasite *Edovum puttleri* Grissell, *E. puttleri* releases alone, or untreated controls. Treatments on beans were similar except the larval parasite *Pediobius foveolatus* (Crawford) was released instead of *E. puttleri.*

Treatments in which *B.t.t.* was used, either alone or in combination with the parasite, significantly reduced adults and 3rd and 4th instar larvae of both species of beetles. The egg parasite, *E. puttleri,* was unable to significantly reduce the CPB population by itself, however *P. foveolatus* was able to significantly reduce both 3rd and 4th instar larval and adult populations of the MBB.

Key Words: Colorado-potato-beetle, Mexican-bean beetle, *Edovum-puttleri, Pediobius-foveolatus,* integrated-control, integrated-pest-management, *Leptinotarsa-decemlineata, Epilachna-varivestis.*

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INTRODUCTION

Strains of *Bacillus thuringiensis* var. *thuringiensis (B.t.t.)* that produce the beta-exotoxin are able to control both the Colorado potato beetle (CPB) and the Mexican bean beetle (MBB) with weekly spray applications containing less than 20 g/acre active ingredient (Cantwell and Cantelo 1982, 1984a, b). Laboratory and field tests however have shown that a level of exotoxin capable of producing 100% mortality of 1st and 2nd instar larvae is not as effective against 3rd and 4th instar larvae and has very little effect against adults except for a slight reduction in feeding and a slightly reduced life span.

In order to seek a higher level of mortality against the beetle populations, parasitic wasps of the family Eulophidae were released into the test plots. *Edovum puttleri,* Grissell an egg parasite of the CPB was used in tomato plots and *Pediobius foveolatus* (Crawford) a larval parasite of the MBB, was used in bean plots.

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Previous feeding tests (unpublished data) indicated that *B.t.t.* has no adverse effects on adults of either of these parasite species.

MATERIALS AND METHODS

The *B.t.t.* material used in these field tests contained 1.8% active ingredient (thuringiensis) w/w and was furnished by Abbott Laboratories and identified as ABG-6162A.[3] Water dilutions of ca 4.7 l per ha. were applied at a pressure of 31.6 kg per sq. cm with a tractor-towed sprayer equipped with drop nozzles. Six applications were made, the first on 31 May 84 and the last on 24 Jul.

In each test, tomatoes and bush snap beans were planted in plots consisting of 5 rows, 10 meters in length and 1.5 m apart. The tomatoes were planted 1 m apart in the row and the beans 5 cm apart. Tomatoes were of the Campbell-28 cv; beans were cv. Provider. Four different treatments were made in the CPB infested plots: *B.t.t.* spray alone, *B.t.t.* spray plus *E. puttleri* releases, *E. puttleri* releases alone, and untreated controls. Each treatment was replicated 3 times. Treatments were similar for the MBB test except *P. foveolatus* was the parasite used.

Two releases of *E. puttleri* were made in the tomato plots; 200 adults per replicate on 4 June and again on 10 July. A single *P. foveolatus* release was made on 26 June at the rate of 1000 adults per replicate.

Weekly counts made beginning 31 May of four different beetle life stages, (1st and 2nd instar larvae, 3rd and 4th instar larvae, adults, and egg masses) were used to determine the efficacy of the treatments. These weekly counts were made in four randomly selected groups of five contiguous tomato plants per replicate and four groups of 1.5 linear meters each of bean plants per replicate. The Waller-Duncan Bayesian K-ratio t test was used to compare means (K = 100) between treatments (Waller and Duncan 1969). Comparisons were made both weekly and for the entire season.

RESULTS AND DISCUSSION

Colorado potato beetle tests. Past studies (Cantwell and Cantelo 1981) have shown that the beta-exotoxin is primarily effective by killing the 1st and 2nd instar larvae. This fact was borne out again throughout the 10-wk period of the present study.

The number of 1st and 2nd instar CPB larvae did not differ significantly between treatments in the summarized data (Table 1) nor were there consistent differences when compared on a weekly basis (Table 2). The treatment combination of *B.t.t.* + parasite resulted in fewer numbers of 1st and 2nd instars as compared to the untreated from the 3rd through the 10th week, however the differences were not always significant. Similar results were obtained in egg mass numbers with no significant differences over the entire testing period. However, from the 5th week on, the *B.t.t.* alone or in combination with *E. puttleri* resulted in significantly fewer egg masses on a week by week basis. Presumably this resulted from reduction of the first generation CPB population by the treatments. The parasite alone was not effective in reducing the numbers of either egg masses or 1st and 2nd instar larvae.

[3] Mention of a commercial (or proprietary) product in this paper does not constitute an endorsement of the product by the U. S. Dept. of Agriculture.

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Table 1. Comparison of treatment means for entire season of life stages of the Colorado potato beetle and the Mexican bean beetle on beans and tomatoes. Organisms used in the treatments were *Bacillus thuringiensis* subsp. *thuringiensis (B.t.t.)* and parasitic eulophid wasps.

	Colorado potato beetle*			
Treatment	Adult	Egg mass	1st & 2nd instar larvae	3rd & 4th instar larvae
B.t.t. + parasite†	0.96 b	2.28 a	5.66 a	0.04 c
B.t.t. alone	0.97 b	2.91 a	10.12 a	0.27 c
parasite alone	2.28 a	3.04 a	8.23 a	7.55 a
untreated	2.93 a	3.00 a	6.22 a	5.29 b
	Mexican bean beetle*			
B.t.t. + parasite‡	0.38 b	0.39 ab	0.85 a	0.06 c
B.t.t. alone	0.43 b	0.33 b	3.80 a	0.58 bc
parasite alone	0.56 b	0.43 ab	4.40 a	2.36 b
untreated	1.00 a	0.62 a	4.65 a	6.00 a

* For each beetle species, means in a column followed by the same letter are not significantly different according to the Waller-Duncan test, K = 100.

† *Edovum puttleri*

‡ *Pediobius foveolatus*

Significant differences were obtained in numbers of both adults and 3rd and 4th instar larvae when *B.t.t.* was applied either alone or in combination with the egg parasite. The reductions resulted from mortality in the early instars. These differences occurred not only over the entire season but almost weekly beginning the 3rd week. However, the parasite alone treatment did not reduce these populations. *Edovum puttleri* had an added effect on reducing all life stage numbers when used in combination with *B.t.t.* although not significantly different from the *B.t.t.* alone treatment.

Mexican bean beetle tests. Striking similarities of results occurred with the MBB treatments compared to like treatments in the CPB tests. The *B.t.t.* alone and in combination with the parasite had no effect on 1st and 2nd instar numbers and no consistent effect on egg mass numbers. In the MBB test, as was discovered in the CPB test, those treatments in which *B.t.t.* was used, a signficant reduction in both 3rd and 4th instar larval and adult populations resulted over the test period (Table 1). The larval parasite of the MBB appeared to be more effective in reducing those latter populations than was the egg parasite of the CPB, having produced significant difference from those of the untreated controls over the entire test period (Table 1).

The *B.t.t.* + parasite combination treatment resulted in consistently fewer numbers than in any of the other treatments of 3rd and 4th instar larvae and adults after the second treatment week (Table 3). Contrary to results with the parasite alone treatment on the CPB, the larval parasite of the MBB, *P. foveolatus* was able to signficantly reduce both 3rd and 4th larval and adult populations of the MBB when compared to the untreated over the entire test period as well as on a weekly basis.

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Table 2. Comparison of weekly mean numbers of Colorado potato beetles by life stages on field plots of tomatoes at Beltsville, MD 1984. Each count represents mean of 5 plants. *Bacillus thuringiensis* subsp. *thuringiensis* = *B.t.t.*; parasite = *Edovum puttleri*. *B.t.t.* applied on weeks # 1, 2, 3, 6, 7, and 9.

Treatment	1	2	3	4	5	6	7	8	9	10
	Number of adults/wk*									
B.t.t. alone	4.08 b	2.08 ab	0.08 b	0.17 b	0.17 b	0.25 c	0.33 b	1.33 a	0.17 b	0.17 b
B.t.t. + parasite	7.09 ab	0.75 b	0.00 b	0.00 b	0.08 b	0.42 c	1.25 b	0.00 b	0.00 b	0.00 b
parasite alone	4.67 b	2.50 a	0.08 b	0.33 ab	0.42 b	7.25 a	3.42 a	1.42 a	1.42 a	1.42 a
untreated	10.25 a	3.00 a	0.75 a	0.75 a	1.17 a	3.83 b	3.42 a	2.00 a	1.58 a	1.58 a
	Number of egg masses/wk*									
B.t.t. alone	6.83 a	8.75 a	0.33 a	0.83 a	0.00 b	0.83 b	1.92 c	2.67 ab	0.75 bc	0.75 bc
B.t.t. + parasite	6.81 a	8.58 a	0.17 a	0.00 a	0.17 b	0.50 b	3.42 bc	0.92 b	0.42 c	0.42 c
parasite alone	4.58 b	6.83 a	0.42 a	0.00 a	0.25 b	1.42 b	5.50 ab	3.83 a	2.17 ab	2.17 ab
untreated	1.92 c	3.08 b	0.33 a	0.42 a	0.83 a	3.83 a	7.67 a	2.83 ab	2.75 a	2.75 a
	Number of 1st & 2nd instar larvae/wk*									
B.t.t. alone	9.41 a	64.92 a	10.50 b	0.00 b	1.00 a	3.50 a	1.58 ab	4.25 a	0.83 b	5.17 a
B.t.t. + parasite	1.64 ab	41.00 b	8.50 b	0.00 b	0.00 a	1.08 a	0.08 b	1.25 a	0.50 ab	2.25 a
parasite alone	0.00 b	19.50 c	41.00 a	3.17 a	0.17 a	0.58 a	1.50 ab	7.67 a	2.67 ab	6.00 a
untreated	0.00 b	11.00 c	13.92 b	1.00 ab	1.33 a	2.33 a	6.67 a	3.83 a	11.00 a	7.25 a
	Number of 3rd & 4th instar larvae/wk*									
B.t.t. alone	0.00 a	0.00 a	0.00 c	0.00 c	0.00 b	2.25 a	0.08 b	0.00 b	0.83 b	0.83 b
B.t.t. + parasite	0.00 a	0.00 a	0.00 c	0.00 c	0.00 b	0.00 a	0.00 b	0.00 b	0.50 b	0.00 b
parasite alone	0.00 a	0.00 a	22.25 a	25.58 a	6.50 a	0.25 a	0.83 b	9.67 a	2.26 ab	1.67 b
untreated	0.00 a	0.00 a	8.75 b	7.75 b	2.08 b	0.58 a	8.25 a	6.50 a	11.00 a	5.58 a

* For each stage, means in a column followed by the same letter are not significantly different according to the Waller-Duncan test, K = 100.

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Table 3. Comparison of weekly mean numbers of Mexican bean beetles on bean plots at Beltsville, MD 1984. (Each count is of 5 row ft). *Bacillus thuringiensis* subsp. *thuringiensis* = *B.t.t.*; *Pediobius foveolatus* = parasite. *B.t.t.* applied on weeks #1, 2, 3, 6, and 7.

Treatment	1	2	3	4	5	6	7
	Number of adults/wk*						
B.t.t. alone	1.00 a	0.33 a	0.58 a	0.17 a	0.33 b	0.25 a	0.33 ab
B.t.t. + parasite	0.83 a	0.50 a	0.33 a	0.25 a	0.50 b	0.17 a	0.00 b
parasite alone	0.92 a	0.83 a	0.33 a	0.50 a	0.42 b	0.33 a	0.58 ab
untreated	0.75 a	1.08 a	1.08 a	0.83 a	1.33 a	0.25 a	1.67 a
	Number of egg masses/wk*						
B.t.t. alone	0.00 a	0.08 a	0.58 a	0.75 ab	0.42 a	0.73 a	0.16 a
B.t.t. + parasite	0.83 a	0.00 a	0.58 a	0.58 b	0.92 a	0.33 a	0.25 a
parasite alone	0.00 a	0.00 a	1.08 a	0.92 ab	0.50 a	0.25 a	0.25 a
untreated	0.00 a	0.33 a	0.92 a	1.50 a	1.08 a	0.33 a	0.17 a
	Number of 1st & 2nd instar larvae/wk*						
B.t.t. alone	0.00 a	0.42 a	0.08 a	10.50 ab	13.58 a	0.58 b	1.42 a
B.t.t. + parasite	0.00 a	0.17 a	0.41 a	1.25 b	1.08 b	0.00 b	3.00 a
parasite alone	0.00 a	0.00 a	0.17 a	16.75 a	5.42 ab	5.17 a	3.33 a
untreated	0.00 a	0.00 a	0.08 a	18.41 a	6.83 ab	3.83 a	3.41 a
	Number of 3rd & 4th instar larvae/wk*						
B.t.t. alone	0.00 a	0.83 a	0.00 b	0.50 ab	0.42 b	1.33 b	1.00 b
B.t.t. + parasite	0.00 a	0.00 a	0.00 b	0.00 b	0.00 b	0.08 b	0.33 b
parasite alone	0.00 a	1.33 a	0.67 ab	1.67 ab	2.17 b	9.50 b	0.67 b
untreated	0.00 a	2.67 a	1.08 a	2.17 a	8.17 a	24.08 a	4.83 a

* For each stage, means in a column followed by the same letter are not significantly different according to the Waller-Duncan test, K = 100.

Over the entire season *B.t.t.* alone reduced the number of CPB adults by 67% and the number of the older larvae by 95%. (The adults and older larvae are the only stages that cause significant losses). When the *B.t.t.* was used with the parasite, the reduction of adults was 67% and of older larvae 99%. The data suggest that *E. puttleri,* when used with *B.t.t.*, makes only a modest contribution to CPB control.

The season-long population of adult MBB was reduced by 57% using *B.t.t.* alone and the population of the older larvae by 90%. Using both the *B.t.t.* and the parasite the adult population was reduced by 62% and the older larvae population was reduced by 99%. The parasite *B.t.t.* combination appeared to have a greater effect on the population of the MBB than the population of the CPB.

Satisfactory control of pest insects, other than lepidopterans, with microbials is uncommon. Control of coleopterans is rare. We have demonstrated that the use of a parasite with a microbial can improve the control achieved by either alone. In the tests with the MBB, both bio-control agents provided good control but together they provided excellent control. It would be expected that further studies would identify other combinations of agents that could have important applications.

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