U. S. Department of Agriculture - Agricultural Research Service

Potential for *Bacillus thuringiensis* as a Biological Control Agent Against Larvae of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) Infesting Florida Citrus

A. A. Weathersbee III, Y. Q. Tang, H. Doostdar, R. T. Mayer
Subtropical Insects Research Unit
U.S. Horticultural Research Laboratory
2001 South Rock Road
Fort Pierce, FL 34945
USA

AWeathersbee@ushrl.ars.usda.gov

Introduction

The most economically important insect pest of citrus in Florida is the root weevil, *Diaprepes abbreviatus* (Figure 1). Developing larvae are subterranean and do extensive damage to host plant root systems and predispose the tissues to pathogenic infections such as *Phytophthora spp*. Larval feeding induces a decline in tree health and fruit production, and may eventually kill the tree. In severe cases where there are uncontrolled weevil populations, entire citrus groves are destroyed. The weevil also causes large economic losses in sugar cane and other horticultural crops in Florida. Control measures for this pest are few and provide only limited population suppression. We have observed significant mortality in laboratory-reared *D. abbreviatus* larvae fed a prepared diet containing spores and δ-endotoxin of *Bacillus thuringiensis*. A search for naturally-occurring *B. thuringiensis* resulted in the isolation of several potentially new strains from diseased *D. abbreviatus* larvae collected in south Florida citrus groves. Florida *B. thuringiensis* isolates are being investigated for novelty and pathogenic activity.

Figure 1. Adults of *D. abbreviatus* apparently are not affected by treatment with *B. thuringiensis var. tenebrionis*.

Figure 2. Healthy (left) and diseased (right) larvae and pupae of *D. abbreviatus* due to treatment with *B. thuringiensis var. tenebrionis*.

Table 1. Mortality of *D. abbreviatus* larvae exposed at 5-weeks old to diet containing *B. thuringiensis var. tenebrionis*.

Treatment (ppm AI)[1]	Percent Mortality (± SE, n=5)[2]
0.0	16.2±5.6a
0.3	28.2±3.2b
3.0	40.4±5.8c
30.0	53.9±5.7d
300.0	67.9±3.8e

Project Activities

Commercial *B. thuringiensis*. We evaluated a commercial preparation (Novodor 3%, Abbott Laboratories, North Chicago, IL) of the microbial agent, *B. thuringiensis var. tenebrionis*, for potential biocontrol of *D. abbreviatus* larvae. Bioassays of diet incorporated with spores and δ-endotoxin of the bacterium indicated that weevil larvae are susceptible to infection (Figure 2). Significant mortality was observed among larvae treated with dietary concentrations as low as 0.3 ppm (Tables 1 and 2). Unlike most reported *B. thuringiensis* activity, mortality in *D. abbreviatus* typically occurred during later larval instars and pupal stages of development. The mortality of larvae exposed to treated diet as neonates was negligible due to high control mortality and variability (data not shown), but the effect on larval weight gain was dose dependent and significant (Table 3). Also, survival was lower among neonate larvae exposed to potted-citrus treated with *B. thuringiensis var. tenebrionis*. Since entomopathogenic nematodes are the only biological agents currently available for root weevil suppression and acceptable control is not always achieved, *B. thuringiensis* would be a welcome addition to root weevil management programs in citrus and other affected crops.

[1]AI refers to the concentration of spores and δ-endotoxin in the prepared diet.
[2]Larval mortality was assessed after five months. Means within a column followed by the same letter are not significantly different (P>0.05).

Table 2. Mortality of *D. abbreviatus* larvae exposed at 12-weeks old to diet containing *B. thuringiensis var. tenebrionis*.

Treatment (ppm AI)[1]	Percent Mortality (± SE, n=4)[2]
0.0	8.3±4.8a
0.3	27.5±11.3b
3.0	38.3±16.8b
30.0	58.3±12.0d
300.0	72.5±10.0e

[1]AI refers to the concentration of spores and δ-endotoxin in prepared diet.
[2]Larval mortality was assessed after three months. Means within a column followed by the same letter are not significantly different (P>0.05).

Table 3. Weight of *D. abbreviatus* larvae exposed as neonates to diet containing *B. thuringiensis var. tenebrionis*.

Treatment (ppm AI)[1]	Weight (mg) (± SE, n=3)[2]
0.0	306.3±22.4a
0.3	251.8±16.7ab
3.0	236.1±21.5ab
30.0	231.1±20.5b
300.0	144.0±20.9c

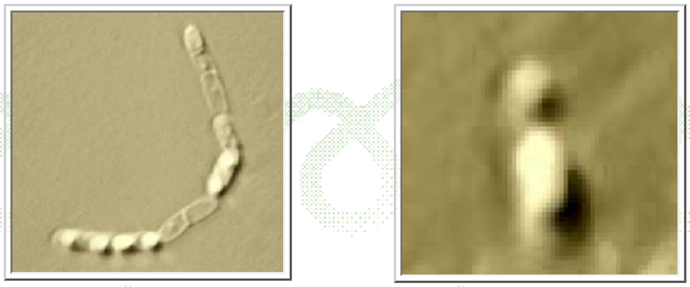
[1] AI refers to the concentration of spores and δ-endotoxin in prepared diet.
[2] Larval weight was assessed after six weeks. Means within a column followed by the same letter are not significantly different (P>0.05).

Table 4. Survival of *D. abbreviatus* larvae exposed as 20 neonates each to potted citrus treated with *B. thuringiensis var. tenebrionis*.

Conc. of suspension (ppm AI)[1]	Number living (± SE, n=3)[2]
0.0	4.17±1.14a
3.0	1.67±0.33b
30.0	1.50±0.43b
300.0	1.33±0.33b

[1]AI refers to the concentration of spores and δ-endotoxin in two 50 ml suspensions applied 14 days apart.
[2] Larval survival was assessed after six weeks. Means within a column followed by the same letter are not significantly different (P>0.05).

Florida *B. thuringiensis* isolates. A search was initiated during February 2000 for naturally-occurring strains of *B. thuringiensis* that infect *D. abbreviatus* larvae (Figure 3). A total of 266 bacterial isolates were selected from samples of soil and diseased weevil larvae collected from Florida citrus groves. We recently developed three sets of novel universal primers for detecting Cry 7, 8, and 9 protein genes in *B. thuringiensis*. Using PCR, we have demonstrated the presence of at least six different classes of Cry protein genes in genomic DNA extractions from isolates in our collection. Examples are provided in Figures 4 and 5. Those identified as having Cry protein genes potentially active against Coleoptera are bioassayed for activity against *D. abbreviatus* larvae.



Isolate No. 96　　　Isolate No. 263

Figure 3. Examples of Florida isolates of B. thuringiensis isolated from diseased larvae of D. abbreviatus. Photomicrographs show spores and δ-endotoxin crystals from isolates nos. 96 and 263.

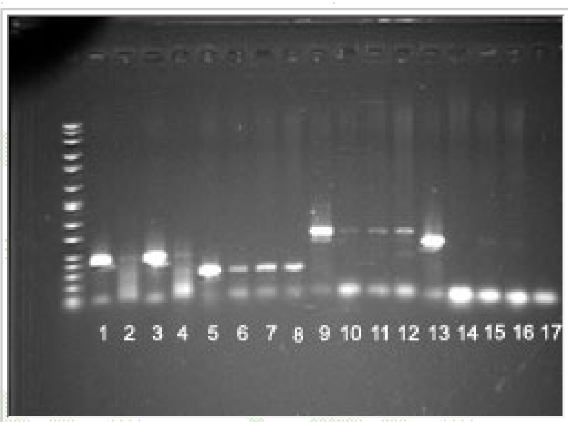


Figure 4. Agarose gel electrophoresis of PCR products amplified from genomic DNA of Florida isolate no. 264. Lanes 5 and 9 are positive controls for Cry 1 and 2 universal primers. Products shown in lanes 6-8 and 10-12 indicate the presence of putative Cry 1 and 2 δ-endotoxin genes in isolate no. 264. Lane 17 is a negative control.

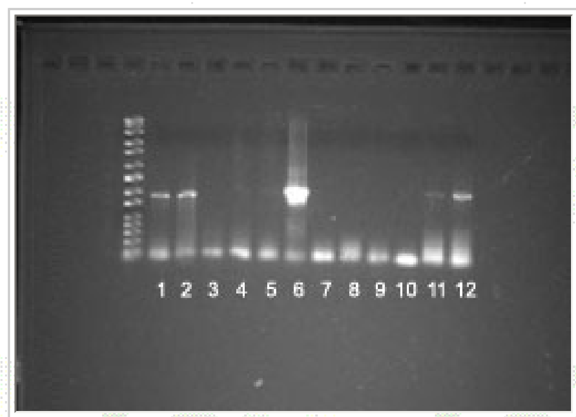


Figure 5. Agarose gel electrophoresis of PCR products amplified from genomic DNA of Florida isolates no. 61 and no. 264. Lane 6 is the positive control for a novel Cry 9 universal primer. Products shown in lanes 1-2 and 11-12 indicate the presence of putative Cry 9 δ-endotoxin genes in both isolates. Lane 7 is a negative control.

Back

Last modified: 8/7/2002. Send comments or questions to Allen Weathersbee at AWeathersbee@ushrl.ars.usda.gov