

Impact of Surfactants on Control of the Root Weevil *Diaprepes abbreviatus* Larvae with *Steinernema riobravisi*¹

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Abstract: A filter paper bioassay was developed for testing the efficacy of nematodes and other agents for control of *Diaprepes abbreviatus* larvae. Surfactants, with and without *Steinernema riobravisi*, were screened first in the filter paper bioassay and then in a potted citrus seedling study. The results of the two assays were in agreement on the relative merits of the compounds tested. Surfactants increased larval mortality at 4 days for the filter paper bioassay and 1 week for the potted plant bioassay. At 8 days for the filter paper bioassay and 2 weeks for the potted plant bioassay, nematodes alone were equal to treatments of nematodes plus surfactants.

Key words: bioassay, biological control, citrus, *Diaprepes abbreviatus*, entomopathogenic nematode, nematode, organosilicone, *Steinernema riobravisi*, Steinernematidae, surfactant.

The root weevil *Diaprepes abbreviatus* (L.) is an important pest of sugarcane, citrus, and ornamentals in the West Indies (Wolcott, 1933). In 1964, *D. abbreviatus* was first detected in the United States in central Florida. By 1995, 25,000 ha of citrus as well as 94 commercial citrus and ornamental nurseries throughout the state were infested (Hall, 1995). The subterranean root-feeding larvae are the primary cause of plant injury and decline; the adults feed on foliage and cause only minor damage. Spread of this exotic weevil from the original infestation near Apopka, Florida, was probably as larvae in soil, adult weevils with plants, egg masses on leaves, and, to a limited extent, flying adult weevils (Beavers and Selhime, 1978; Schroeder and Beavers, 1977).

Rhabditid nematodes of the family Steinernematidae and Heterorhabditidae are lethal to a broad range of economically important insect pests (Gaugler and Kaya, 1990; Poinar, 1971). Several species have been evaluated for control of *D. abbreviatus* larvae in soil with encouraging results (Schroeder, 1987b, 1992). Recently, *Steinernema riobravisi*

(Cabanillas et al., 1994) was isolated from the Lower Rio Grande Valley in Texas, parasitizing the corn earworm *Helicoverpa zea* (Boddie) and the fall armyworm *Spodoptera frugiperda* (J. F. Smith) (Raulston et al., 1992). *Steinernema riobravisi* was more effective than *S. carpocapsae* Weiser as a biological control agent against *D. abbreviatus* larvae (Schroeder, 1994).

The effect of pesticides on nematodes used for the biological control of insect pests has been thoroughly evaluated (Gaugler and Campbell, 1991; Ishibashi and Takii, 1993; Rovesti and Deseo, 1990; Zimmerman and Cranshaw, 1990). In this study surfactants used to improve the efficacy of pesticides were examined. Surfactants also have been used to increase efficacy of biological control agents, including increased penetration of bacteria into weeds for biological control (Zidack et al., 1992). Insect control of an aphid also was enhanced by the use of surfactants (Imai et al., 1994). Efficacy of biological control agents is usually less than that obtained with conventional pesticides. To attempt to increase the efficacy of nematodes, *Diaprepes* larvae were exposed to nematodes in a solution containing a surfactant. This study compares the efficacy of *S. riobravisi* with and without the addition of a surfactant for control of *D. abbreviatus* larvae.

MATERIALS AND METHODS

Nematodes: *S. riobravisi* that had been reared in vivo in *H. zea* were obtained from

H. E. Cabanillas, USDA, ARS, Weslaco, Texas. The nematodes were stored on expanded foam in T-flasks at 10 °C.

Surfactant toxicity to nematodes: Ten ml of distilled water with 7,000 nematodes was placed in each of four 200-ml beakers. The toxicity of three surfactants—Agri-SC (Four Star, Inc., Bluffton, IN), Silwet L-77 (Union Carbide Chemical and Plastic Co., Danbury, CT), and Tegoprene 5840 (Goldschmidt Chem. Corp., Hopewell, VA)—were tested by adding them at a concentration of 0.05% (v/v). No surfactant was added to the control. Mortality was assessed at 1 and 7 days under a microscope to determine nematode infection.

Weevil larvae: *D. abbreviatus* were reared on diet (Beavers, 1982) and were 3 weeks old when used in the laboratory filter paper bioassay and 4 months old in the potted seedling study (Schroeder, 1987a). The average weight of the larvae was 0.06 g for 3-week-old larvae and 0.4 g for 4-month-old larvae.

Laboratory filter paper bioassay: Filter paper 3 cm in diameter was placed in 3.5-cm petri dishes. Filter paper was soaked with 300 µl water containing the following treatments: water only; 200 infective juveniles (IJs) of nematodes; 200 IJs of nematodes plus surfactants at 0.1% (v/v) for the dishwash detergent (Dawn, Proctor and Gamble, Cincinnati, OH), 0.05% (v/v) for the organosilicone surfactants Silwet L-77, Tegoprene 5840, and Kinetic (Helena Chem. Co., Memphis, TN), and 3% (w/v) of the water retention compound carboxymethylcellulose (Cell-U-Wet Creative Services, Michigan City, IN). Each treatment was with and without nematodes. Artificial diet (1 cm³) was added to each petri dish. Each treatment was replicated five times, with one larva per dish and 10 dishes per replicate. Dishes were incubated in the dark at 32 °C in a moisture chamber at 90% humidity. Mortality was recorded at 4 and 8 days.

Potted plant bioassay: Sour orange (*aurantium* L.) seedlings were planted in 10-cm diameter pots filled with 2 liters of peat and one part coarse sand (v/v). Plants were maintained in the shade under ambient conditions at 25 °C and watered as needed. Ten *D. abbreviatus*

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Potted plant bioassay: Sour orange (*Citrus aurantium* L.) seedlings were planted in 15-cm diameter pots filled with 2 liters of three parts Florida peat and one part coarse builder's sand (v/v). Plants were maintained outside under ambient conditions at 22–30 °C and watered as needed. Ten *D. abbreviatus*

larvae were placed 10 cm below the soil surface of each pot. After 1 week, the following treatments were added to the pots in 25 ml of distilled water: *S. riobravisi* at the rate of 9 nematodes/cm³ of soil, 0.05% ammonium laureth sulfate (Agri-SC) and the organosilicone surfactants (Silwet L-77, Tegoprene 5840, and Kinetic), 0.1% dishwashing detergent (Dawn), and 3% (w/v) of carboxymethylcellulose (Cell-U-Wet). All treatments were with and without nematodes. The soil was removed from the pots after 1 week, and the number of live larvae determined. The test was repeated except that the duration was increased to 2 weeks with the water control, nematodes alone, and all compounds with nematodes except for Cell-U-Wet, dishwashing detergent, and Kinetic. The tests were conducted over a 1-year period with 20 plants per treatment. Data were transformed with arcsin (\sqrt{x}) and subjected to an analysis of variance (ANOVA), and means were separated with the Student-Newman-Keuls multiple-range test. Data are presented using the original scale of measurement.

RESULTS

Surfactant toxicity to nematodes: The surfactants were not toxic to the nematodes ($P = 0.05$). Control mortality was 7% at 1 day and 12% at 7 days. With the addition of surfactants, nematode mortality ranged from 6% to 10% at 1 day, and from 8% to 12% at 7 days.

Laboratory bioassay: Surfactants alone did not affect insect mortality after 4 days (Table 1). Surfactants with nematodes gave the highest mortality, ranging from 60% to 70%. Intermediate levels of insect mortality resulted from nematodes alone (32%) and nematodes and Tegoprene 5840 (34%) (Table 1). At 8 days, there were similar mortalities (90% to 100%) between the nematode treatments with and without surfactants. Tegoprene 5840 alone caused 26% mortality (14% for Dawn) followed by the other surfactants, and 2% for the controls.

Potted plant bioassay: No differences in insect mortality resulted from potted plants treated with surfactants alone or the control

TABLE 1. Effects of surfactants and/or *S. robravis* nematodes on the mortality of *Diaprepes abbreviatus* larvae in the filter paper bioassay 4 and 8 days after treatment.

Treatment	% Mortality	
	4 Days	8 Days
Control	2 a	2 a
Cell-U-Wet	0 a	4 ab
Dawn Dish Detergent	6 a	14 b
Kinetic	2 a	6 ab
Silwet L-77	2 a	6 ab
Tegoprene 5840	12 a	26 c
Nematodes	32 b	90 d
Nematodes and Cell-U-Wet	68 c	98 d
Nematodes and Dawn Dish Detergent	60 c	92 d
Nematodes and Kinetic	66 c	98 d
Nematodes and Silwet L-77	70 c	100 d
Nematodes and Tegoprene 5840	34 b	98 d

Means ($n = 5$) within the same column followed by the same letter are not significantly different ($P = 0.05$) according to the Student-Newman-Keuls multiple-range test.

($P = 0.05$) (Table 2). Mortality for the control was 10% at 1 week and 17% at 2 weeks; larval mortality in plants treated with surfactants ranged from 2% to 14% at 1 week.

TABLE 2. Effects of surfactants and/or *S. robravis* nematodes on the mortality of *Diaprepes abbreviatus* larvae in soil 7 and 14 days after treatment as observed in the potted plant bioassay.

Treatment	% Mortality	
	1-Week Exposure	2-Week Exposure
Control	10 a	17 a
Agri-SC	2 a	
Cell-U-Wet	9 a	
Dawn Dish Detergent	14 a	
Kinetic	10 a	
Silwet L-77	9 a	
Tegoprene 5840	10 a	
Nematodes	25 b	69 b
Nematodes and Agri-SC	58 c	71 b
Nematodes and Cell-U-Wet	16 ab	
Nematodes and Dawn Dish Detergent	56 c	
Nematodes and Kinetic	54 c	
Nematodes and Silwet L-77	47 c	85 c
Nematodes and Tegoprene 5840	24 b	67 b

Means ($n = 20$) within the same column followed by the same letter are not significantly different ($P = 0.05$) according to the Student-Newman-Keuls multiple-range test.

When ammonium laureth sulfate (Agri-SC) and organosilicones (Silwet L-77 and Kinetic) were added to the nematode suspension at 0.05% and dishwashing detergent (Dawn) was added at 0.1%, larval mortality was significantly higher after 1 week compared to treatments with nematodes only. Tegoprene 5840 at 1 week did not increase larval mortality. Mortality averaged 47% to 58% for other surfactants with nematodes and 25% for nematodes only. Mortality averaged 67% to 85% at 2 weeks with nematodes and surfactants and 69% with nematodes only. The differences were significant ($P = 0.05$) after 1 week, but only mortality of larvae in pots treated with Silwet L-77 was higher after 2 weeks. Carboxymethylcellulose (Cell-U-Wet) at 3% (w/v) had no effect on mortality of *D. abbreviatus* larvae in soil.

DISCUSSION

Surfactants alone did not cause significant mortality of *D. abbreviatus* larvae. Surfactants had no effect on nematode viability at 1 and 7 days in solution. Both the filter paper and potted plant assays showed greater differences between treatments at the shortest time interval. Control mortality in the filter paper bioassay was most likely the result of handling. Control mortality of *D. abbreviatus* larvae in the pot bioassay was probably the result of cannibalism by other larvae and other natural mortality factors in pots maintained outside. These results suggest that surfactants may increase initial penetration of nematodes into the larvae. After longer exposure periods, the nematodes apparently are able to penetrate the larvae and the difference in mortality is about equal. Only Silwet L-77 had a significant effect after 2 weeks in potted citrus.

The results of the two assays are comparable. The filter paper bioassay is a rapid, reproducible, and simple bioassay to screen nematode preparations for the biological control of *D. abbreviatus* larvae. The previous sand bioassay used larger larvae, took 4 weeks, and gave more variable results (Schroeder, 1992). The potted plant assay simulates *D. abbreviatus* infestations that oc-

cur in citrus and multi-species nurseries. These surfactants were evaluated at the recommended application rate for soil and not considered environmental contaminants. Cell-U-Wet increased mortality in the filter paper bioassay but not in the potted citrus study. In the pots, it tended to accumulate on the soil surface and this probably reduced penetration of the soil surface by nematodes. Four of the five surfactants when included in the nematode tank increased mortality of *D. abbreviatus* larvae. These compounds are routinely applied in citrus and multi-species nurseries to increase the efficacy of pesticides and are especially available to the grower. Nematodes should be applied with surfactants in nurseries as they can be applied cheaply (1000 acre). When *Diaprepes* larvae are killed, root damage is minimized. In this situation, the *D. abbreviatus* larvae are confined to pots, and nematodes are applied through the irrigation system. This project did not address the use of surfactants in conjunction with grove irrigation. Additional studies are needed to evaluate the ability of surfactants to increase the efficacy of nematodes for controlling *Diaprepes* larvae under grove conditions.

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