Effect of Neem Seed Extract on Feeding, Growth, Survival, and Reproduction of *Diaprepes abbreviatus* (Coleoptera: Curculionidae)

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ABSTRACT A commercially available neem seed extract, Neemix 4.5, containing 4.5% azadirachtin (AZA), was assessed for biological activity against the root weevil *Diaprepes abbreviatus* (L.), an important exotic insect pest of Florida citrus. Laboratory bioassays against neonatal and 3-wk-old larvae fed sliced carrot treated with Neemix produced dose-dependent larval mortality and reduced fresh weights among survivors of treatments. The weight response was greater than the mortality response for both larval age groups. Neonates treated with 45 mg/liter AZA weighed 60% less than those in the control after 4 wk. Three-week-old larvae treated with 45 mg/liter AZA weighed 30% less than those in the control after 5 wk. When neonates were exposed to insect diet incorporated with Neemix, reductions in larval survival and weight were observed at concentrations as low as 4.8 mg/liter AZA after 6 wk. Larval growth was inhibited by >97% with 42.9 mg/liter AZA in the diet. A soil drench containing 30 mg/liter AZA reduced the survival and weight gain of neonates added to potted citrus and provided protection to the roots in a greenhouse experiment. A concentration of 90 mg/liter AZA was required to provide protection of citrus roots against 4-wk-old larvae. Reproductive effects were observed when adult weevils were fed foliage treated with Neemix. The numbers of larvae hatching per egg mass were reduced by 27% and 68% at 30 and 90 mg/liter AZA, respectively. These results suggest that Neemix should be further evaluated for use in integrated pest management (IPM) programs of citrus.

KEY WORDS *Diaprepes abbreviatus,* root weevil, neem extract, citrus, Neemix, azadirachtin.

THE USE OF natural biochemical pesticides in commercial agricultural and horticultural industries has increased in recent years. These bio-pesticides offer desirable alternatives to using synthetic chemicals in agricultural systems where protection of the environment and preservation of beneficial organisms are important. One such bio-pesticide of interest is the natural insect growth regulator (IGR), azadirachtin (AZA), a botanical compound that can be effective, is biodegradable, and rapidly metabolizes in the environment (Isman 1999). This compound is a liminoid that accumulates in the seeds of the neem tree (*Azadirachta indica* A. Juss.), from which it can be extracted efficiently (Butterworth and Morgan 1968, Schroeder and Nakanishi 1987). Crude formulations of neem seed extracts also contain other liminoids that contribute to insecticidal properties (Mordue (Luntz) and Blackwell 1993). The diverse effects of AZA on insect pests include feeding deterrence, reproduction disturbance, and insect growth regulation among others (Mordue (Luntz) et al. 1998, Walter 1999). Furthermore, the compound apparently has minimal toxicity to nontarget organisms such as parasitoids, predators, and pollinators (Lowery and Isman 1995, Naumann and Isman 1996) increasing its acceptability for control of phytophagous insects both to pest managers and regulatory agencies.

Over 400 insect pests have been shown to exhibit varying degrees of susceptibility to neem seed extracts, or the most active constituent AZA (Schmutterer and Singh 1995); yet, pests of citrus are not well represented among those evaluated. Jacobson (1981) reported that a methanol soluble fraction of neem seed extract was repellent to adults of the root weevil *Diaprepes abbreviatus* (L.). This exotic insect has become a well-established pest of citrus in Florida since it was first discovered in 1964 (Woodruff 1964). Effective control measures for *D. abbreviatus* are lacking and the infested acreage continues to increase. Annual losses caused by the pest are in excess of $75 million due to decreased fruit production, cost of control, and replanting expenses (Anonymous 1997). Because of the threat *D. abbreviatus* poses to future citrus production in Florida, we evaluated a commercially avail-

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able neem seed extract (Neemix 4.5) for biological activity against this pest.

Materials and Methods

Insect Source and Rearing. Insects used in the experiments were obtained from a colony of *D. abbreviatus* maintained by the U.S. Horticultural Research Laboratory, Fort Pierce, FL. Larvae were reared on a commercially-prepared, insect diet (Product No. F1675, Bio-Serv, Frenchtown, NJ) placed within sealed cups (30 ml cups with lids). Methods similar to those of Beavers (1982) were used to prepare insect diet and rear larvae to adults. Temperature and moisture content of diet were optimized for larval development according to Lapointe (2000) and Lapointe and Shapiro (1999).

Biopesticide and Plant Sources. A formulated neem seed extract (Neemix 4.5 [4.5% AZA]) was obtained from Thermo Trilogy, Columbia, MD. The product was screened at various concentrations against *D. abbreviatus* larvae and adults to evaluate effects on feeding, growth, survival, and reproduction. Citrus plants used in the root feeding experiments were Swingle citrumelo [*Citrus paradisi* Macf. × *Poncirus trifoliate* (L.) Raf.] and those used in leaf feeding and oviposition experiments were Cleopatra Mandarin (*Citrus reshni* Hort., ex. Tan.). Both citrus varieties were 1-yr-old seedlings potted in 140-cm^3 containers with potting soil (Metromix 500, Scotts, Marysville, OH).

Laboratory Bioassays. An experiment was conducted to determine biological effects of Neemix against neonatal larvae of the *D. abbreviatus.* Suspensions containing Neemix were prepared at 0, 11.3, 22.5, 45, 90, and 180 mg/liter AZA in DI water. Carrot slices (≈1.5 cm diameter × 2 mm thick) were soaked in the treatment suspensions for 10 min before use. A treatment consisted of 30 neonate larvae, placed singly in capped diet cups containing a slice of treated carrot. Treatments were replicated three times. Weevil larvae were maintained on treated carrot for 5 d and then transferred to untreated carrot slices for an additional 23 d. Carrot slices were replaced every 3–5 d as needed due to larval feeding and degradation. Larval mortalities and the fresh weights of survivors were recorded at the end of the study.

A similar experiment was conducted against 3-wk-old larvae. Larvae used in the study were ≈20 mg each before exposure to treatments. Treated carrot slices were prepared as above. A treatment consisted of 24 3-wk-old larvae, placed singly with a slice of treated carrot in the wells of a 24-well, cell culture tray with lid. Treatments were replicated three times. Weevil larvae were maintained on treated carrot for 5 d and then transferred to untreated carrot slices for an additional 27 d. Carrot slices were replaced as needed and larval mortalities and the fresh weights of survivors were recorded at the end of the study.

The effect of extended exposure of neonates to insect diet incorporated with Neemix was evaluated. Prepared insect diet was heated to 90°C for 15 min, covered with foil, and allowed to cool to 56°C in a heated water bath before incorporating Neemix. The product was incorporated into the diet at rates of 0.0, 1.6, 4.8, 14.3, 42.9, and 128.6 mg/liter AZA. Treatments were incorporated into the diet with the aid of a heated, stirrer plate and the resulting mixtures were pipetted into diet cups (15-ml diet per cup) and allowed to solidify. Ten neonate larvae were placed in each diet cup and a lid was affixed. There were 30 replicates of each treatment. All steps after heating of the diet were performed in a laminar flow, clean bench to avoid contamination. Larval survival and the fresh weights of survivors were determined after 6 wk.

Greenhouse Trials. The effect of soil treatment with Neemix was evaluated in separate experiments against neonates and 4-wk-old larvae feeding on the roots of citrus seedlings. Suspensions of Neemix were prepared at concentrations of 0, 10, 30, and 90 mg/liter AZA in DI water. Neonate larvae (20 per plant) were placed on the surface of the soil in each citrus seedling container and covered with 1 cm of fine sand. The bottom of each container was wrapped with parafilm and aluminum foil to prevent egress of larvae. After allowing 24 h for weevil larvae to become situated, soil drench treatments were applied as 30-ml suspensions to each container (12 replications of each treatment). The plants were maintained in a small greenhouse and watered (30 ml) twice weekly. The numbers and fresh weights of surviving larvae, and dry weights of the total and adventitious root systems of each plant were recorded after 6 wk.

Four-week-old larvae (one per plant) were placed 2 cm beneath the soil surface in each citrus seedling container. Larvae used in the test were ≈34 mg each. An additional control treatment was included that contained no larvae. After allowing 24 h for weevil larvae to become situated, the soil in each container was drenched with 30 ml of suspension. Each treatment, comprising 10 plants, was replicated three times. Treatments were maintained and watered, and data were recorded as above after 6 wk.

Choice greenhouse experiments were conducted to determine if *D. abbreviatus* adults preferred feeding and ovipositing on foliage of citrus either treated or untreated with Neemix. Application rates were 10, 30, and 90 mg/liter AZA in DI water. Three cages were used in the experiment corresponding to the three treatment rates. Each cage contained two bouquets (treated and untreated) of flushing citrus foliage as food, two containers (treated and untreated) each with six nonflushing citrus seedlings as oviposition sites, and 10 pairs of newly emerged adult weevils acclimated by feeding on clean foliage for 1 wk. Treatments were applied via a hand-held sprayer. The citrus bouquets and seedlings were sprayed until runoff and allowed to air dry before they were placed in the cages. Untreated foliage and seedlings were sprayed with water and handled similarly. Feeding effects were measured after 2 d by scoring the area of foliage consumed from treated and untreated bouquets (0 = 0%; 1 = 1–25%; 2 = 26–50%; .3 = 51–75%; and 4 = 76–100%). Effects on oviposition were measured by counting the number of egg masses deposited on treated and untreated seedlings after 2 d. The experiment was replicated eight times.

No-choice greenhouse experiments were conducted to determine if feeding Neemix-treated citrus foliage to adult *D. abbreviatus* affected either oviposition behavior or egg viability. Application rates were 0, 10, 30, and 90 mg/liter AZA in DI water. Four cages were used in the experiment corresponding to the four treatment rates. Each cage contained a bouquet of treated, flushing citrus foliage; four artificial oviposition substrates (two treated and two untreated) fashioned from waxed paper strips (Wolcott 1933); and 10 pairs of newly emerged, acclimated adult weevils. The untreated waxed paper strips were included to determine if potential effects on egg viability were due to disruption of reproductive physiology in adult weevils fed treated foliage or toxicity to eggs caused by contact with treated surfaces. Citrus foliage and waxed paper strips were sprayed to runoff with the appropriate AZA concentrations and allowed to air dry before they were placed in cages. Untreated foliage and waxed paper strips were sprayed with water. Effects on oviposition were measured by counting the number of egg masses deposited on treated and untreated waxed paper strips, and effects on fecundity were measured by monitoring larval hatching. Treated foliage bouquets and waxed paper strips were replaced every 2 d to generate data for 10 sample dates. The experiment was replicated three times.

Data Analyses and Statistics. Data were analyzed by the General Linear Models Procedure, and differences among treatment means were determined by Tukey's studentized range test (SAS Institute 1990). Percentage data were adjusted for control mortality using the Abbott (1925) formula and transformed (arcsine) before analyses. Differences among means were considered significant at a probability level of 5% ($P \leq 0.05$). Untransformed means were presented in the data tables.

Results

Laboratory Bioassay on Neonates. The effect of Neemix-treated carrot on mortality of neonate larvae was significant ($F = 5.48$; df = 5, 9; $P = 0.0137$), but the dose–response was weak. A maximum of 40% mortality was observed at the highest concentration tested (180 mg/liter AZA). However, a highly significant ($F = 64.43$; df = 5, 131; $P < 0.0001$) dose-dependent reduction in larval growth was observed (Table 1) indicating that treatments caused feeding deterrence or growth regulation. The fresh weights of treated larvae were significantly less ($P \leq 0.05$) than those of control larvae at all concentrations tested.

Laboratory Bioassay on Three-Week-Old Larvae. The effect of Neemix-treated carrot on mortality of 3-wk-old larvae also was significant ($F = 4.62$; df = 5, 10; $P = 0.0191$), but the dose–response was weak and similar to that observed for neonate larvae. The highest concentration tested (180 mg/liter AZA) provided only 25% mortality. The reduction in larval weights due to treatments was significant ($F = 26.53$; df = 5, 10; $P < 0.0001$) and dose-dependent (Table 2), indicative of antifeedancy or growth regulation. The fresh weights of larvae treated at concentrations ≥22.5 mg/liter were significantly reduced ($P \leq 0.05$) as compared with the controls.

Diet Incorporation Bioassay on Neonates. Neonates fed an insect diet containing Neemix exhibited significant reductions in both survival ($F = 35.61$; df = 5, 144; $P < 0.0001$) and growth ($F = 18.08$; df = 4, 79; $P < 0.0001$). Both larval survival and weight gain decreased in a dose-dependent manner with increasing AZA concentration. A concentration of only 4.8 mg/liter AZA provided significant reductions ($P \leq 0.05$) in larval survival and weight compared with the controls (Table 3). The low survival rate for neonates in the control group is addressed in the discussion section. The weight of larvae in the 4.8 mg/liter treatment was >60% less than that of control larvae. Larval growth was almost completely inhibited in the 42.9 mg/liter treatment. No larvae survived 6 wk of exposure to diet containing 128.6 mg/liter AZA.

Greenhouse Trials with Neonates. Applications of Neemix as a soil drench to citrus roots resulted in significant reductions in survival ($F = 6.60$; df = 3, 32; $P = 0.0013$) and fresh weights ($F = 11.24$; df = 3, 25; $P < 0.0001$) of larvae exposed as neonates for a period

Table 1. Percent mortality and average fresh weight of *D. abbreviatus* larvae exposed as neonates to carrot slices treated with Neemix for 5 d, then to untreated carrot slices until day 28

Treatment (mg/liter azadirachtin)[a]	% mortality ± SE ($n = 90$)[b]	Weight (mg) per surviving larvae ± SE (n)[b]
0.0	2.4 ± 1.2b	24.3 ± 1.1a (88)
11.3	2.5 ± 2.5b	17.5 ± 0.9b (87)
22.5	13.3 ± 6.9ab	13.0 ± 0.8c (78)
45.0	12.4 ± 4.6ab	9.6 ± 0.6cd (79)
90.0	25.3 ± 5.8ab	7.5 ± 0.6de (67)
180.0	40.0 ± 13.3a	5.0 ± 1.0e (54)

[a] Each treatment comprised 30 neonate larvae and was replicated three times.
[b] Means within a column sharing the same letter were not significantly different ($P > 0.05$, Tukey's studentized range test [SAS Institute 1990]).

Table 2. Percent mortality and average fresh weight of *D. abbreviatus* exposed as 3-wk-old larvae to carrot slices treated with Neemix for 5 d, then to untreated carrot slices until day 32

Treatment (mg/liter azadirachtin)[a]	% mortality ± SE ($n = 72$)[b]	Weight (mg) per surviving larvae ± SE (n)[b]
0.0	0.0 ± 0.0b	70.0 ± 1.0a (72)
11.3	6.9 ± 1.4ab	64.3 ± 2.4ab (67)
22.5	8.3 ± 4.8ab	51.3 ± 0.3bc (66)
45.0	8.3 ± 4.8ab	49.0 ± 0.6c (66)
90.0	13.9 ± 5.6ab	44.3 ± 4.4cd (62)
180.0	25.0 ± 4.2a	32.3 ± 3.5d (54)

[a] Each treatment comprised 24 3-wk old larvae and was replicated three times. Larvae used in the test weighed ≈20 mg each before exposure to treatments.
[b] Means within a column sharing the same letter were not significantly different ($P > 0.05$, Tukey's studentized range test [SAS Institute 1990]).

Table 3. Survival and average fresh weight of *D. abbreviatus* larvae exposed as neonates for 6 wk to insect diet incorporated with Neemix

Treatment (mg/liter azadirachtin)[a]	No. of surviving larvae per diet cup ± SE (n = 300)[b]	Weight (mg) per surviving larvae ± SE (n)[b]
0.0	3.62 ± 0.33a	75.3 ± 8.2a (105)
1.6	3.60 ± 0.29a	58.7 ± 7.3a (105)
4.8	2.23 ± 0.37b	27.1 ± 5.0b (67)
14.3	2.10 ± 0.28b	21.0 ± 2.9b (63)
42.9	0.37 ± 0.11c	1.8 ± 0.6b (11)
128.6	0.00 ± 0.00c	—

[a] Each treatment comprised 10 neonate larvae per diet cup and was replicated 30 times.
[b] Means within a column sharing the same letter were not significantly different (P > 0.05, Tukey's studentized range test [SAS Institute 1990]).

Table 5. Initial fresh weights and average weight gains for *D. abbreviatus* exposed as 4-wk-old larvae, and average dry weights of citrus adventitious roots, six weeks after treatment with a soil drench (30 ml per plant) containing Neemix

Treatment (mg/liter azadirachtin)[a]	Initial weight (mg) per larvae ± SE (n = 30)[b]	Weight gain (mg) per surviving larvae ± SE (n)	Adventitious root weight (mg) ± SE (n = 30)
0.0	34.9 ± 1.0a	231.6 ± 8.2a (30)	114.5 ± 19.5c
10.0	34.6 ± 1.0a	231.7 ± 10.6a (30)	150.8 ± 22.2c
30.0	33.8 ± 0.9a	213.1 ± 8.0a (29)	291.9 ± 55.7c
90.0	33.5 ± 1.0a	163.5 ± 14.3b (20)	603.3 ± 65.6b
0.0 (no larvae)	—	—	826.1 ± 57.0a

[a] Each treatment comprised one larvae per plant and was replicated 30 times.
[b] Means within a column sharing the same letter were not significantly different (P > 0.05, Tukey's studentized range test [SAS Institute 1990]).

of 6 wk. Significant increases in the dry weights of citrus adventitious roots (F = 12.55; df = 3, 32; P < 0.0001) and whole roots (F = 4.75; df = 3, 32; P < 0.0075) were observed for the treated as compared with control plants, indicating that damage from neonatal larvae was reduced by treatments. Data were presented only for adventitious roots because the F-statistics, R-square values, and coefficients of variation were greater than those for whole roots.

Of 20 neonates initially infesting each plant, nine larvae were recovered on average from the control plants after 6 wk, whereas only 4.6 larvae were recovered from plants treated with 30 mg/liter AZA (Table 4). The low recovery rate for larvae in the control group is addressed in the discussion section. Surviving larvae in all treatments weighed significantly ($P \leq$ 0.05) less than those in the control, indicating that treatments applied as a soil drench caused larval antifeedancy or growth regulation. The dry weights of citrus adventitious roots in treatments ≥30 mg/liter weighed significantly ($P \leq 0.05$) more than those of the controls, demonstrating that soil applications of Neemix protected citrus roots from feeding damage caused by neonates.

Greenhouse Trials with Four-Week-Old Larvae. Applications of Neemix as a soil drench on 4-wk-old larvae infesting potted citrus caused significant reductions in weight gains of surviving larvae (F = 8.15; df = 3, 103; P < 0.0001). There were no significant differences in the initial weights of larvae used in the experiment (F = 0.45; df = 3, 114; P = 0.7192). Treatments also caused significant increases in the dry weights of adventitious roots (F = 40.93; df = 4, 143; P < 0.0001) and whole roots (F = 30.98; df = 4, 143; P < 0.0001).

Table 4. Survival and average fresh weight of *D. abbreviatus* exposed as neonate larvae, and average dry weights of citrus adventitious roots, 6 wk after treatment with a soil drench (30 ml per plant) containing Neemix

Treatment (mg/liter azadirachtin)[a]	No. of surviving larvae ± SE (n = 240)[b]	Weight (mg) per surviving larvae ± SE (n)[b]	Adventitious root weight (mg) ± SE (n = 12)
0.0	9.00 ± 1.05a	25.4 ± 1.7a (108)	152.5 ± 55.8c
10.0	6.00 ± 1.08ab	16.8 ± 1.6b (72)	306.7 ± 41.3bc
30.0	4.58 ± 1.20b	14.6 ± 2.8bc (55)	489.9 ± 60.5ab
90.0	2.25 ± 0.62b	8.2 ± 1.6c (27)	601.0 ± 55.6a

[a] Each treatment comprised 20 larvae plant and was replicated 12 times.
[b] Means within a column sharing the same letter were not significantly different (P > 0.05, Tukey's studentized range test [SAS Institute 1990]).

The weight gains of surviving larvae in the 90 mg/liter treatment were 30% less than those of control larvae (Table 5). The dry weights of adventitious roots in the 90-mg/liter treatment were five times those of the untreated controls, but were less than those in the noninfested control ($P \leq$ 0.05). In treatments that received <90 mg/liter AZA, the fresh weights of larvae and dry weights of citrus roots were not significantly different (P > 0.05) from those in the infested controls indicating that larvae beyond the neonatal stage were less susceptible to the effects of treatments (Table 5). A reduction in larval survival (30%) was observed only in the 90 mg/liter treatment (data not included in table).

Choice Tests with Adult Weevils. In choice tests, adult weevils demonstrated no feeding preference for treated (3.2 ± 0.1) or untreated (3.0 ± 0.2) citrus foliage (F = 2.19; df = 1, 39; P = 0.1466) as determined by scores of feeding damage. The effects of application rate (F = 1.37; df = 2, 38; P = 0.2656) and the rate by treatment interaction (F = 2.54; df = 2, 14; P = 0.1144) also were not significant regarding the amount of foliage consumed. This indicated that Neemix did not act as a repellent or feeding inhibitor against adult *D. abbreviatus* within the concentration range tested.

Adult weevils displayed no oviposition preference for treated (8.8 ± 0.8 egg masses) or untreated (10.9 ± 0.9 egg masses) foliage (F = 3.87; df = 1, 39; P = 0.0563) although the F-statistic was nearly significant. Interestingly, more egg masses were deposited as the concentration of AZA increased (F = 6.74; df = 2, 38; P = 0.0031). The number of egg masses deposited by weevils exposed to 90 mg/liter AZA was 35% greater than those deposited by weevils exposed to 10 mg/liter AZA (Table 6). We observed that egg masses in the

Table 6. Numbers of egg masses deposited from caged adult *D. abbreviatus* given a choice of six citrus seedlings treated with Neemix and six untreated citrus seedlings as oviposition sites

Treatment (mg/l azadirachtin)[a]	No. of egg masses per six seedlings ± SE ($n = 16$)[b]	Choice (treated or untreated oviposition sites)	Number of egg masses per six seedlings ± SE ($n = 8$)[b]
10.0	7.81 ± 0.72c	T	7.75 ± 1.11b
		U	7.88 ± 0.99b
30.0	9.69 ± 0.79ab	T	9.38 ± 1.35b
		U	10.00 ± 0.93b
90.0	12.06 ± 1.40a	T	9.38 ± 1.87b
		U	14.75 ± 1.70a

T, treated; U, untreated.
[a] Each treatment comprised 10 pairs of adult weevils per cage and was replicated eight times.
[b] Means within a column sharing the same letter were not significantly different ($P > 0.05$, Tukey's studentized range test [SAS Institute 1990]).

90-mg/liter treatment appeared discolored and misshapen. Since this was an indication of potential reproductive effects we attempted to further address this observation in no-choice tests. The rate by treatment interaction was significant ($F = 4.98$; df = 2, 14; $P = 0.0233$) and revealed that more ($P \leq 0.05$) egg masses were oviposited on untreated foliage at the highest application rate but not at the lower rates (Table 6). Adult weevils may be able to avoid treated foliage as oviposition sites when application rates are high and untreated foliage is available.

No-Choice Tests with Adult Weevils. When adult weevils were fed treated foliage under no-choice conditions, they oviposited equal numbers of egg masses on treated and untreated waxed paper strips ($F = 1.69$; df = 1, 235; $P = 0.1947$) indicating that Neemix did not act as an oviposition deterrent. Also, larvae hatched in equal numbers per waxed paper strip ($F = 0.33$; df = 1, 235; $P = 0.5678$) and per egg mass ($F = 0.18$; df = 1, 235; $P = 0.6687$) on treated and untreated strips demonstrating that Neemix was not toxic to encased *D. abbreviatus* eggs.

In contrast to results from the choice test, the concentration of AZA on treated foliage provided under no-choice feeding conditions did not affect the numbers of egg masses oviposited on waxed paper strips ($F = 1.38$; df = 3, 233; $P = 0.2499$) (Table 7). Differences in the oviposition substrates (cuticularized leaf versus waxed paper) used in the two experiments may have altered oviposition behavior and caused the discrepancy. Of greater importance was the finding that the numbers of larvae that hatched per strip ($F = 19.81$; df = 3, 233; $P < 0.0001$) and per egg mass ($F = 34.62$; df = 3, 233; $P < 0.0001$) significantly declined with increasing AZA concentration, indicating that treatments impaired the reproductive physiology of adult weevils or were transferred by the adults to embryonic tissues resulting in reduced egg development. A 27% reduction ($P < 0.05$) in larval hatch per waxed paper strip was observed at the lowest concentration (10 mg/liter). Larval hatch per strip was reduced by 35% at 30 mg/liter, and 63% at 90 mg/liter (Table 7). The numbers of larvae hatching per egg mass were reduced by 27% and 68% in the 30- and 90-mg/liter treatments, respectively. The dose-dependent reductions in egg viability we observed were attributed to reproductive effects in adult weevils that fed on treated foliage.

The effect of sample date was significant with respect to the number of egg masses deposited per waxed paper strip ($F = 4.60$; df = 9, 227; $P < 0.0001$), number of larvae hatched per strip ($F = 4.84$; df = 9, 227; $P < 0.0001$) and number of larvae hatched per egg mass ($F = 2.54$; df = 9, 227; $P = 0.0085$) indicating that treatment effects varied over time. Nevertheless, the *F*-statistics for the split-effect (date) were small relative to those for the main effect (concentration), implying that the effect of date was minor relative to the effect of concentration.

Table 7. Numbers of egg masses deposited per egg strip and measures of egg viability from caged adult *D. abbreviatus* fed citrus foliage treated with Neemix in no-choice tests

Treatment (mg/liter azadirachtin)[a]	Egg masses per strip ± SE ($n = 60$)[b]	Live larvae per strip ± SE ($n = 60$)[b]	Live larvae per mass ± SE ($n = 60$)[b]
0.0	12.87 ± 0.79a	526.70 ± 39.92a	40.05 ± 2.31a
10.0	11.55 ± 0.82a	385.55 ± 33.64b	33.52 ± 1.94ab
30.0	11.73 ± 0.60a	340.42 ± 28.39b	29.13 ± 2.16b
90.0	13.32 ± 0.91a	192.78 ± 25.76c	12.66 ± 1.51c

[a] Each treatment comprised 10 pairs of adult weevils per cage and was replicated three times.
[b] Means within a column sharing the same letter were not significantly different ($P > 0.05$, Tukey's studentized range test [SAS Institute 1990]).

Discussion

Our laboratory evaluations of the biological effects of Neemix indicated that survival rates of *D. abbreviatus* larvae were reduced after ingesting treated food, most notably among those exposed as neonates. However, a greater effect of treatment was the observed reduction in weight gain, particularly when larvae were treated as neonates. The effect on weight gain appeared to be due to growth regulation since feeding ceased only in treated larvae just before death. The reductions in weight gain observed in *D. abbreviatus* larvae were in agreement with the findings of Schlüter (1985), who reported that treatment by injection with AZA inhibited the production of a fatbody storage protein, resulting in weight gain reductions for the Mexican bean beetle, *Epilachna varivestis* Mulsant. Feeding deterrence cannot be ruled out as a possible effect of AZA on *D. abbreviatus* larvae since we did not measure larval feeding in these experiments, but we observed that all larvae continued to feed until signs of toxicity were evident. Mordue (Luntz) et al. (1996) reported a similar condition for the locust *Locusta migratoria* (R & F), which would ingest enough treated plant foliage to cause toxic physiological effects while another locust *Shistocerca gregaria* (Forskål) would starve before feeding on treated foliage.

The low survival rates we observed for neonates in our control groups are typical in experiments such as this, where multiple larvae are used to challenge plants or generate data for bioassays of diet-incorporated materials. Low survival rates for neonatal *D. abbreviatus* have been reported by others (Schroeder and Sieburth 1997, Quintella and McCoy 1997) and are due to natural mortality factors including hostile interactions among larvae confined together (Lapointe and Shapiro 1999).

The *D. abbreviatus* larval period is highly variable and can range from 3 to 18 mo under colony rearing conditions (Lapointe and Shapiro 1999). Wolcott (1934) estimated the total developmental cycle to be ≈1 yr in the field while Beavers (1982) reported a cycle of a little more than 1 yr when larvae were reared in the laboratory on artificial diet. Our laboratory assays of Neemix on treated carrot slices lasted ≈30 d while the diet incorporation and soil drench assays were terminated after 6 wk, so the full effect of treatments on the insect's ability to complete a life cycle was not determined. The growth reductions seen for survivors of treatments may be an indication of toxic physiological effects that caused mortality in later developmental stages of other insects (Mordue (Luntz) and Blackwell 1993). Given the long and inherently variable life cycle for this weevil, it is reasonable to assume that the developmental effects observed in treated larvae would increase their susceptibility to natural controls. An evaluation of the effect of Neemix treatment on life cycle completion by *D. abbreviatus* is warranted.

The results of our greenhouse experiments indicated that the survival of root feeding, neonatal larvae in the soil could be reduced by applying root drenches containing 30 mg/liter AZA. This rate also provided protection of the plant root system against neonate larvae during the 6-wk period of the experiment. A 90-mg/liter treatment was required for protection of plant roots infested with 4-wk-old larvae, indicating that control of larval stages beyond neonatal will be more difficult to achieve. Nevertheless, our experiment was 6 wk in duration and used a single, soil application to target 4-wk-old larvae that were established in the soil. Multiple applications targeting neonates during the egg-laying season may prevent development of larvae to stages that cause damage. Xie et al. (1991) previously demonstrated that soil drenches of AZA protect the roots of corn plants from attack by the corn rootworm, *Diabrotica virgifera virgifera* (LeConte) and also reported assimilation and systemic movement of the compound within the plant. Given that systemic activity of AZA has been reported in other plants (Knodel et al. 1986, Nisbet et al. 1993), relevance to the protection of citrus roots from larval feeding by *D. abbreviatus* are worth investigating.

Root weevils create an additional problem in citrus because the damage caused by larval feeding predisposes otherwise healthy plant roots to infections by pathogens such as *Phytophthora* spp. (Rogers et al. 1996). The use of Neemix to protect citrus roots from damage by weevil larvae should also reduce opportunities for pathogenic infection. Furthermore, Mordue (Luntz) and Blackwell (1993) reported on the activity of neem extracts against several plant pathogenic fungi; however, no reference was made to *Phytophthora* spp. The potential affects of Neemix on the *D. abbreviatus-Phytophthora* interaction should be investigated.

Neem-based products have been reported to cause toxicity and growth regulation effects in other coleopteran insects (Ladd et al. 1984, Schlüter 1985, Schmutterer and Singh 1995, Trisyono and Whalon 1999) similar to those reported here. We also observed that larval age influenced the susceptibility of *D. abbreviatus* to Neemix, as was previously reported for *Coccinella septempunctata* L. larvae (Banken and Stark 1997). Our findings that Neemix did not act as a repellent or feeding deterrent to adult *D. abbreviatus* under choice conditions were in contrast to that of Jacobson (1980), who reported repellent activity of a methanol soluble fraction of neem extract against adults. The difficulties associated with standardization of neem products due to multiple constituents and multiple analogs of azadirachtin have been previously discussed (Isman 1999, Mordue (Luntz) and Blackwell 1993).

The effects on egg viability that we observed were due to feeding by adult weevils on treated foliage and not contact of eggs with a treated surface. This indicates that treatments caused physiological disruption of the reproductive cycle, an important and powerful effect that has been discussed by others (Karnavar 1987, Mordue (Luntz) and Blackwell 1993). Although population suppression may not be immediately perceptible in long-lived insects such as *D. abbreviatus*, disruption of reproductive capacity can cause substantial population decline over time.

Neem-based insecticides have been found to have little impact on many beneficial organisms such as pollinators, predators and parasitoids (Lowery and Isman 1995, Naumann and Isman 1996, Walter 1999). It appears that Neemix is compatible with IPM in the citrus ecosystem where protection of natural enemies is sought. Our results indicated that Neemix applied as a soil drench reduced the survival and weight gain of *D. abbreviatus* neonates and provided protection to infested citrus roots.

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