HORTICULTURAL ENTOMOLOGY

Effects of Imidacloprid on Development, Mobility, and Survival of First Instars of *Diaprepes abbreviatus* (Coleoptera: Curculionidae)

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ABSTRACT The effect of imidacloprid on mortality and ecdysis of 1st instars of *Diaprepes abbreviatus* L. was determined using contact and oral exposure bioassays. The effect of contact exposure to imidacloprid on 1st-instar mobility was determined with and without a soil substrate. Greenhouse studies were conducted to determine possible systemic effects of different rates of imidacloprid applied as a soil drench on larval mortality and on root protection of container-grown citrus seedlings. When larvae were fed treated carrot or artificial diet at doses of ≥12.5 ppm, imidacloprid reduced feeding, which contributed to slower larval development and reduced ecdysis. Imidacloprid also affected larval mobility and development by contact exposure at doses >100 ppm. Larval mobility was impaired significantly in soil treated with imidacloprid at doses >6 μg/g of soil. In moistened soil, larval movement was significantly impaired at 6 μg/g of soil. Mortality caused by imidacloprid was slow for either mode of entry but was 6 times faster by oral than by contact exposure. When exposure to doses of imidacloprid was followed by periods without exposure to the chemical, larvae were able to recover. When imidacloprid was applied as a soil drench to container-grown citrus trees at 200 ppm, no larvae survived in the soil, suggesting death from starvation before reaching plant roots or death after feeding on roots containing imidacloprid.

KEY WORDS *Diaprepes abbreviatus*, Admire, soil insect, behavior modification, soil insect control

Diaprepes abbreviatus L., a root weevil native to the Lesser Antilles of the Caribbean region, has emerged as a major localized pest of citrus and ornamental plants in Florida since its introduction in 1964 (Woodruff 1964, Woodruff 1985, McCoy and Simpson 1994). Adult weevils feed on young leaves, but larval feeding on fibrous roots and the bark of primary roots, particularly those in the crown region, causes citrus tree decline or death (Wolcott 1936). An estimated 40,000 ha of citrus, or ≈12% of the total commercial area in Florida, are infested with *D. abbreviatus* and at least one-half of this hectarage is exhibiting severe decline or is out of production (Hall 1995).

For many years, chlorinated hydrocarbons were applied as a soil barrier beneath the tree canopy for control of 1st instars of *D. abbreviatus* (Bullock 1985). Following their cancellation, organophosphates and carbamates were used but resulted in lesser control. Because of groundwater contamination risks, insecticidal usage gradually declined during the past decade (McCoy and Simpson 1994). Currently, no pesticides are used for larval control in the field, but considerable research is underway to find new cultural, biological, and chemical management tools.

Imidacloprid, a nitromethylene heterocycle analogue, is a broad-spectrum insecticide effective against many sucking insects and several species of Coleoptera, Diptera, and Lepidoptera found in the soil (Elbert et al. 1991). It is systemic in plants and has a neurotoxic mode of action in insects (Abbink 1991). Imidacloprid has low mammalian toxicity (Elbert et al. 1991, Liu and Casida 1993) and degrades rapidly in the soil (Schroeder and Flattum 1984).

Imidacloprid is somewhat unique in that it appears to enhance the pathogenicity of some entomopathogenic fungi occurring in the soil. Baits containing imidacloprid have caused high mycosis to termites in natural soil containing either *Metarhizium anisopliae* (Metschnikoff) Sorokin or *Conidiobolus coronatus* (Costantin) Batko (B. J. Monke, personal communication). Furthermore, strong synergism was detected against eastern subterranean termites, *Reticulitermes flavipes* (Kollar), when sublethal doses of imidacloprid were added

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to soil containing conidia of *M. anisopliae*, *Paecilomyces farinosus* (Dickson), *C. coronatus*, *Beauveria bassiana* (Balsamo) Vuillemin, and *Actinomucor* sp. (B. J. Monke, personal communication).

In an attempt to develop a microbial control strategy for 1st instars of *D. abbreviatus* at the soil surface, a mycelial formulation of *M. anisopliae* and a conidial formulation of *B. bassiana* have been field tested in Florida (McCoy 1991, Schwarz 1995). These studies showed that high inoculum levels were required to achieve efficacious levels of mycosis and that inoculum persistence was highly variable. In view of these findings and those of B. J. Monke, we conducted a series of experiments to determine the effect of different doses of imidacloprid on 1st-instar mortality, larval ecdysis, and mobility. We evaluated contact and oral exposure, with and without a soil substrate, as a precursor to further studies using entomopathogenic fungi with imidacloprid.

Materials and Methods

Insect. First instars of *D. abbreviatus*, <48 h old, were used in all experiments. Larvae were obtained from eggs laid by field-collected females confined to screened cages in the greenhouse at 27 ± 2°C as described by McCoy et al. (1995). Before each experiment, the most vigorous larvae were selected for experimentation by placing them at one end of a plastic column (14 cm in length and 3.0 cm in diameter) through a 3-mm-diameter hole, by using a small funnel. This device was enclosed in black paper to prevent light from entering the column. At the opposite end of the column, a fiberoptics light source was positioned ≈15 mm away to attract the larvae through the column. As the larvae moved toward the light, they fell through an opening made in the middle of the column into a 50-ml beaker, which was used to support the column. Vigorous larvae were defined as those traveling 7 cm in 30 min.

In all bioassays, larvae were held at 28°C in the dark in 30-ml clear plastic cups containing a 1.5-cm-diameter filter paper disc moistened in sterile distilled water and a thin slice of raw carrot as a food source. The filter paper was moistened every other day and the carrots replaced when they were desiccated or contaminated with bacteria or fungi.

Chemical. A 21.4% formulation of Admire 2 F (flowable), technical grade imidacloprid 1-[(6-chloro-3-pyridinylmethyl)]-*N*-nitro-2-imidazolidin-imine, was supplied by Bayer (Kansas City, MO) for use in all experiments.

Soil. The soil used in the bioassays was classified as a Candler fine sand (Typic quartzipsamments, hyperthermic, uncoated) with a particle size distribution of 96.7% sand, 0.8% silt, and 2.5% clay. The organic matter content was 0.80%, bulk density 1.47 g/cm^3, and the pH 5.7.

Statistical Analyses. Regression analyses for the bioassays were performed on proportional data transformed to arcsine square root by using the general linear models procedure unless stated otherwise (SAS Institute 1985).

Larval Survival and Development Following Contact and Oral Exposure to Imidacloprid. Three separate bioassays were conducted to determine the effect of exposure method and dose of imidacloprid on larval survival and development. In test 1, formulated imidacloprid diluted in sterile distilled water to concentrations of 0, 12.5, 25, 50, and 100 ppm (AI) was tested using 3 exposure methods. Cohorts of 5 first instars were dipped for 5 s in the chemical solution and then placed on filter paper to dry. Larvae were treated orally by feeding them throughout the experiment on small pieces of treated carrot, previously dipped in chemical for 5 min and dried on filter paper. The 3rd treatment was a combination of contact and oral exposure methods. Each treatment was replicated 5 times. Live and dead larvae were recorded daily for 10 d by microscopic examination at 10–16× magnification. Head capsule width of living larvae was measured microscopically at 50× magnification at the termination of the test.

In test 2, the experimental protocol was the same as for test 1 except an artificial diet was substituted for carrot and experimental results recorded 23 d after treatment. The artificial diet was prepared according to the procedures reported in McCoy et al. (1985). Two hundred and fifty microliters of each concentration of imidacloprid was pipetted onto the surface of the diet.

In test 3, first instars were treated by placing ≈70 individuals in 1.5-ml microcentrifuge tubes and gently agitating them for 30 s in imidacloprid solutions of 0, 50, 100, 250, 500, and 1,000 ppm (AI). Larvae were fed imidacloprid throughout the experiment on small pieces of carrot that were immersed in the chemical for 30 min. Each treatment was replicated 5 times with 10 larvae per replication. Live and dead larvae as well as larval ecdysis were recorded by microscopic examination every other day for 9 d.

Larval Mobility After Contact Exposure to Imidacloprid. Cohorts of 60 larvae were treated with imidacloprid at 0, 50, 100, 150, and 200 ppm (AI) by submersion for 30 s in 1.5-ml microcentrifuge tubes. Twenty larvae were placed in the plastic column described previously. The time required for the first 10 larvae to move 7 cm was determined for 4 replicates during a 1-h period. This procedure was conducted at 0, 24, and 48 h after chemical treatment. Treatment means for larval movement in time were analyzed with the SAS general linear models procedure and compared using the Tukey honestly significant difference (HSD) test at $P = 0.05$ (SAS Institute 1985).

Larval Survival, Development, and Recovery After Oral Exposure to Imidacloprid. Pieces of 8-mm-diameter carrot were dipped in imidacloprid solutions of 0, 50, 100, 500, and 1,000 ppm (AI) for 30 min. Larvae were fed treated carrot for

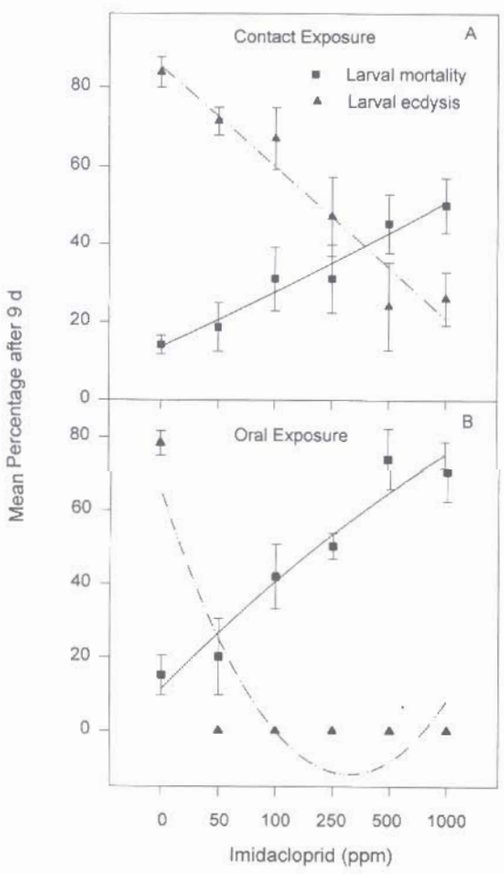

Fig. 3. Effect of oral (A) and contact application (B) of imidacloprid to 1st instars of *D. abbreviatus* on mortality and ecdysis after 9 d.

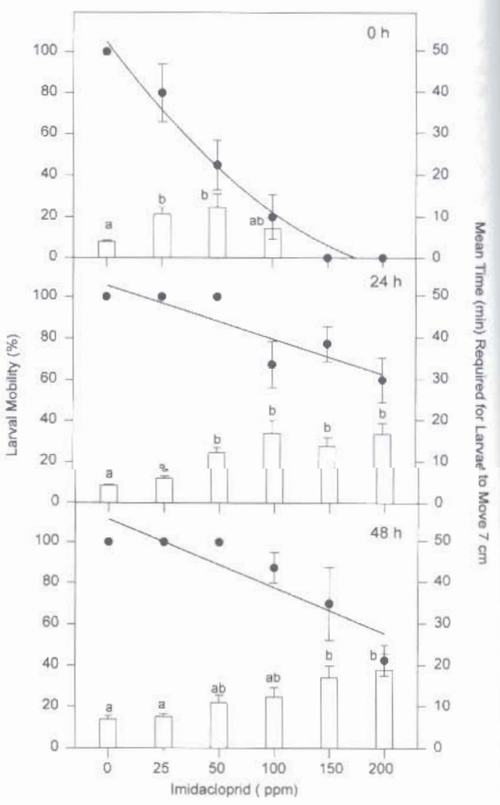

Fig. 4. Mean percentage of larval mobility (dots) and mean time required for larvae of *D. abbreviatus* to move 7 cm (bars), at 0, 24, and 48 h after cuticular treatment with imidacloprid. Bars with the same letter are not significantly different by the Tukey test ($P < 0.05$).

trations of imidacloprid completed normal larval ecdysis (Fig. 2B).

The results for test 3, where doses of imidacloprid at 50–1,000 ppm were applied orally or as a contact treatment to 1st-instar, are presented in Fig. 3. After contact application, larval mortality increased linearly with an increase in imidacloprid dose ($r^2 = 0.37$; $F = 17.41$; df = 1, 28; $P < 0.001$) (Fig. 3B). Even at 1,000 ppm, only 50% of larvae were killed after 9 d. When larvae were fed carrot treated with imidacloprid, however, percentage of mortality in time also increased with an increase in the dose and fit a quadratic model ($r^2 = 0.65$; $F = 13.82$; df = 2, 27; $P < 0.001$) (Fig. 3A). By comparison, percentage of larval mortality after oral exposure was higher at all chemical doses >50 ppm.

All concentrations of imidacloprid ranging from 50 to 1,000 ppm applied orally or to the larval cuticle influenced larval ecdysis (Fig. 3 A and B); in fact, larvae fed imidacloprid on treated carrot failed to molt ($r^2 = 0.37$; $F = 8.76$; df = 2, 27; $P < 0.01$). All doses affected larval behavior within a few hours after feeding. Many larvae stopped feeding and some exhibited abnormal locomotory behavior such as tremors and tumbling. When imidacloprid was applied to the larval cuticle, however, ecdysis decreased with an increase in doses up to 500 ppm and the relationship appeared to best fit the quadratic model ($r^2 = 0.68$; $F = 13.72$; df = 2, 27; $P < 0.001$).

Larval Mobility After Contact Exposure to Imidacloprid. The mobility of 1st instars declined in time with an increase in the concentration of imidacloprid after contact treatment (Fig. 4). A negative quadratic relationship between mobility and chemical dose was found at 0 h and changed to a linear relationship at 24 and 48 h after treatment. Immediately after treatment (0 h), larval mobility decreased significantly at 25, 50, and 100 ppm ($r^2 = 0.88$; $F = 14.55$; df = 2, 21; $P < 0.001$) and larvae were totally immobile at 150 and 200 ppm. At 24 and 48 h after treatment, larval mobility was significantly different among chemical doses ($r^2 = 0.62$; $F = 35.48$; df = 1, 22; $P < 0.0001$), ($r^2 = 0.71$; $F = 47.89$, df = 1, 22; $P < 0.0001$), but mobility increased

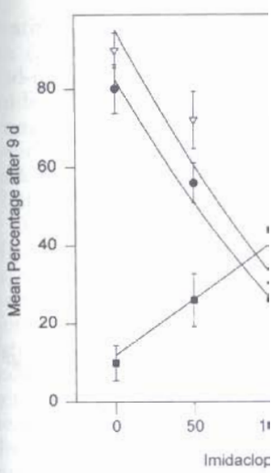

Fig. 5. Effect of oral expo
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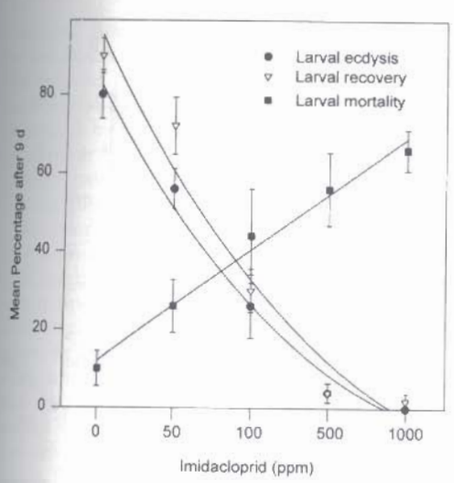

Fig. 5. Effect of oral exposure of 1st instars of *D. abbreviatus* on larval mortality, larval ecdysis, and larval recovery after 9 d. Larvae were considered recovered from imidacloprid treatment if they showed no sluggishness or uncoordinated quivering.

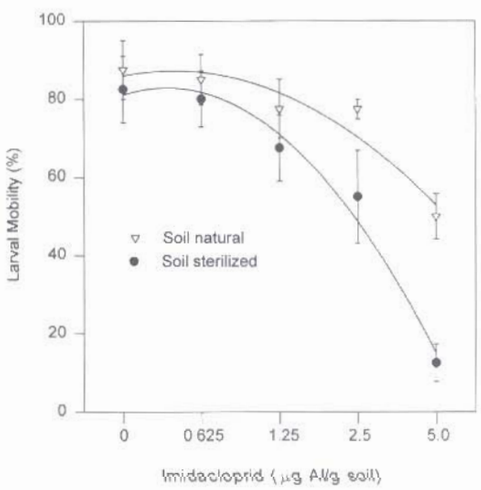

Fig. 6. Effect of imidacloprid on mobility of 1st instars of *D. abbreviatus* in sterilized and natural soil after 48 h.

with time at all doses, suggesting some loss in chemical effect. In fact, at 24 and 48h after treatment, 100% of the larvae exhibited normal movement at doses of 25 and 50 ppm.

Larval mobility measured in time also was affected by chemical doses (Fig. 4). At time zero, larvae treated with imidacloprid at 100 ppm or less required 8–12 min to travel 7 cm compared with 4 min for the control larvae. At higher doses (150–200 ppm) where larvae were immobile at 0 h, they now required 15–18 min to travel the defined distance. Generally, larval mobility in time was significantly affected by imidacloprid at ≥50 ppm.

Larval Survival, Development, and Recovery After Oral Exposure of Imidacloprid. Some 1st instars fed treated carrot for 3 d regained normal activities and even completed molting when fed untreated carrot (Fig. 5). A similar negative quadratic relationship was observed between larval ecdysis and chemical doses ($r^2 = 0.86$; $F = 10.33$; df = 2, 22; $P < 0.004$) and larval recovery and chemical doses ($r^2 = 0.86$; $F = 10.09$; df = 2, 22; $P < 0.004$). These results suggest that larval ecdysis is a good indicator of larval recovery from paralysis from imidacloprid treatment. Larval mortality increased linearly with doses of imidacloprid ($r^2 = 0.60$; $F = 33.96$; df = 1, 23; $P < 0.0001$). However, percentage of larval mortality was less than for test 4 where larvae were fed treated carrots throughout the study at concentrations >500 ppm (Fig. 3B). After replacement of the treated carrots, larval ecdysis was 56 and 26% at 50 and 100 ppm, respectively (Fig. 5), compared with no ecdysis even at 12.5 ppm when larvae were exposed continually to treated carrot (Figs. 1–3).

Larvae Mobility and Survival After Treatment with Imidacloprid to Natural and Sterilized Candler Soil. In test 1, the vertical movement of 1st instars through soil at 12% moisture decreased with an increase in the dose of imidacloprid in both autoclaved ($r^2 = 0.72$; $F = 17.47$; df = 2, 17; $P < 0.001$) and natural soil ($r^2 = 0.53$; $F = 7.38$; df = 2, 17; $P < 0.015$) and best fit a quadratic model in both cases (Fig. 6). Overall, larval movement was lowest in autoclaved soil. When autoclaving and oven drying Candler sandy soil it became electrostatic and probably affected larval movement. At 5 μg/g of soil, only 12.5 and 50% of the larvae moved through both autoclaved and natural soil, respectively.

In test 2, larval mobility in natural Candler soil was not affected by moisture level in the controls (Fig. 7A). However, larval mobility decreased significantly with an increase in dose of imidacloprid at all moistures (Table 1). The relationship between mobility and chemical dose and moisture best fit a quadratic and a linear model, respectively (Table 1). Larval mobility in treated soil appeared to decrease as soil moisture increased. For example, at 6 μg/g of soil, only 22.4% of the larvae were recovered from soil at 12% moisture compared with 67 and 70% recovery at 6 and 2% moisture, respectively.

Larval mortality among those remaining in the soil after 8 d was not significantly affected by soil moisture (Fig. 7B; Table 1). In treated soil, mortality varied significantly for the different doses of imidacloprid; in fact, a dose response was found only at 6% soil moisture.

Larval Mortality and Root Protection to Citrus Seedlings After a Soil Drench with Imidacloprid in the Greenhouse. Imidacloprid at rates of 50, 100, and 200 ppm significantly reduced larval populations compared to the control (Table 2).

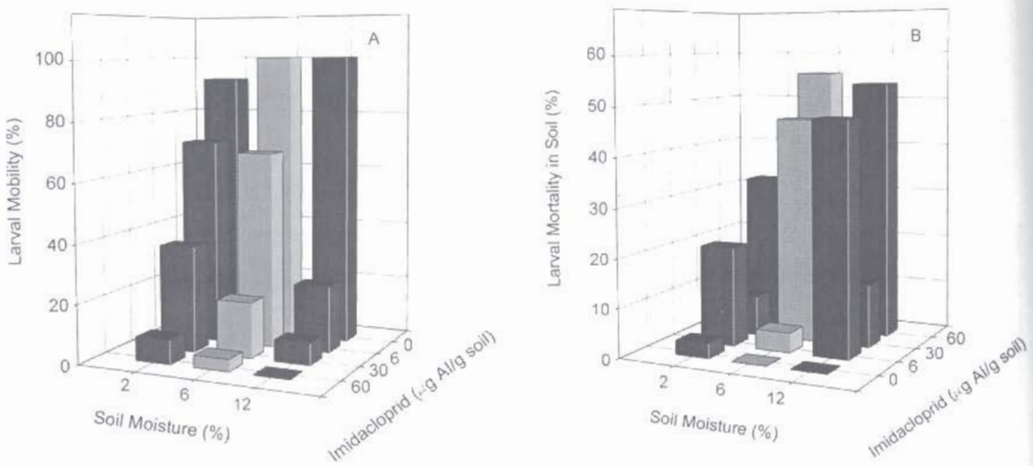

Fig. 7. Effect of imidacloprid on mobility and survival of 1st instars of *D. abbreviatus* in natural Candler soil at different soil moistures after 8 d.

The mean fibrous root mass was significantly lower in all chemical treatments compared with untreated control without larvae. However, fibrous root mass was significantly greater at 200 ppm compared with control (larvae). At 100 and 200 ppm, there was no detectable root injury to the plants, suggesting feeding inhibition, systemic activity, or both at these rates.

Discussion

According to McCoy et al. (1995), imidacloprid appeared to be systemic at doses of 200 ppm and affected development of 1st instars of *D. abbreviatus* by oral exposure at doses of ≥100 ppm. Our studies confirmed these findings. In addition, we showed that larvae stopped feeding after oral exposure at doses as low as 12.5 ppm. Imidacloprid affected larval mobility and development by contact exposure at doses >100 ppm. Larval mobility was impaired significantly in soil treated with imidacloprid at doses >6 μg/g of soil. However, at a higher soil moisture level (12%), larval movement was impaired significantly even at 6 μg/g of soil. In terms of larval mortality, our results substantiate those of McCoy et al. (1995) in that contact exposure in and outside a soil substrate at doses <500 ppm had little effect. However, larvae fed carrot were highly susceptible at doses >100 ppm, suggesting that the 6-fold difference in susceptibility was influenced by chemical penetration of the larval cuticle. Liu et al. (1993) showed that low contact toxicity by imidacloprid was caused by poor cuticular penetration and rapid oxidative detoxification. When imidacloprid was applied as a soil drench to container-grown citrus trees at 200 ppm, no larval survival occurred in the soil. Because larval mortality in treated soil was low at doses <500 ppm, it appears that imidacloprid is systemic in citrus trees and toxic to larvae at low doses. These data also showed that the mode of action for imidacloprid was slow for either mode of entry. For example, 9 d was required to kill 70% of the larvae at 500 ppm by oral exposure and 1,000 ppm was required to kill 50% by contact exposure. This slow mode of action is typical of some neurotoxins and has been reported for imidacloprid against the subterranean termite *R. flavipes* (B. J. Monke, personal communication) and larvae of *Heliothis virescens* (F.) and *Spodoptera littoralis* (Boisduval) (Lagadic and Bernard 1993).

Although we did not quantify feeding behavior, larvae fed either carrot or synthetic diet exhibited reduced mobility and feeding activity. The latter likely contributed to slower development (ecdysis) because 14 ± 5% of larvae without food molted compared with 80 ± 6.3% of larvae with food. No reports of molting inhibition have been reported in the literature for imidacloprid. Antifeedant action caused by imidacloprid has been reported for *R. flavipes* (B. J. Monke, personal communication), *Somaticus* spp. (Drinkwater 1994), and *Heteronychus arator* Fabricius (Drinkwater and Groenewald 1994). However, Lagadic and Bernard (1993) found no evidence of antifeedant action or repellent effect with larvae of *H. virescens* and *S. littoralis* on artificial diet and concluded that restricted feeding was the result of intoxication involving paralysis or motor incoordination of the mouthparts. No paralysis of the larval mouthparts was observed in our study; in fact, excitation of the mouthparts occurred. We did observe abdominal tremors, uncoordinated quivering, erratic head movement, fluid discharge, and a tumbling effect, suggesting possible gut paralysis that would affect digestion of the food.

In our investigation, 1st instars treated topically and orally were able to recover from imidacloprid

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treatment. Boucias et al. (1996) also showed that the termite *R. flavipes* recovered from imidacloprid-induced paralysis after their transference to imidacloprid-free soil. The nitromethylenes can be synergized by inhibitors of oxidative metabolism, cytochrome P450 inhibitors, suggesting that they undergo oxidative detoxification (Schroeder and Flattum 1984, Liu et al. 1993).

Our results showed that exposure of 1st instars of *D. abbreviatus* to soil treated with sublethal doses of imidacloprid resulted in altered mobility, at least temporarily. It would appear that this additional stress could lead to subsequent larval starvation, or the loss of mobility could make larvae more vulnerable to attack by predators and pathogens in the soil. Preliminary data suggest that healthy larvae moving actively in soil will void themselves of infective conidia attached to their cuticle. One can assume that altered behavior will then prevent this process from occurring, thereby making the larvae more vulnerable to infection by fungi and other microbes. Studies are currently underway to test this hypothesis.

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