SOIL SURFACE APPLICATIONS OF CHEMICALS FOR THE CONTROL OF NEONATE *DIAPREPES ABBREVIATUS* (COLEOPTERA: CURCULIONIDAE) AND THEIR EFFECT ON ANT PREDATORS

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ABSTRACT

The root weevil, *Diaprepes abbreviatus,* as a larva, inflicts feeding injury to the bark of all root parts of a citrus tree, thereby impairing root function and supplying infection courts for soil-borne root rot diseases. Ideally, larvae should be controlled at the soil surface before they reach the root zone. In screenhouse and field experiments conducted in central Florida from 1996-99, the synthetic pyrethroid, bifenthrin, at 0.54 g/m^2 (0.554 kg ai/ha) and RPA107382, an analog of fipronil, at 0.156 and 0.312 ml/m^2 (0.242-0.466 kg ai/ha), were applied uniformly to the soil surface beneath the tree to form a chemical barrier against neonates of *D. abbreviatus.* By comparison to the control, larval populations were reduced by 80-100% within one week and these reductions persisted for 4-8 weeks. In an open screenhouse, bifenthrin gave excellent root protection of container-grown trees during a 22 week period when neonates were added to containers weekly for 12 weeks. RPA107382 was highly effective for about 2 weeks but lacked residual effect. The accumulation of leaf litter beneath the tree impaired coverage of the soil by bifenthrin resulting in reduced control. According to weekly baited trap counts, both chemicals reduced non-target foraging ants, particularly *Solenopsis invicta Buren*. The reduction in *S. invicta* was temporary however, but it did allow time for other foraging ants to re-establish and increase.

Key Words: Chemical control, bifenthrin, citrus root weevil, ant predators

RESUMEN

El picudo *Diaprepes abbreviatus*, en etapa de larva, inflige daño al comer de la corteza de todas las partes de la raíz de un árbol cítrico, así perjudicando la función de las raíces y supliendo sitios de infección para enfermedades por suelo de podredumbre radical. Idealmente, las larvas deberían ser controladas en la superficie del suelo antes de alcanzar la zona radical. En invernaderos y experimentos hechos en la Florida central de 1996-99, el piretroide sintético bifenthrin, a 0.54 g/m^2 (0.554 kg ai/ha) y RPA107382, un análogo de fipronil, a 0.156 y 0.312 ml/m^2 (0.242-00.466 kg ai/ha), fueron uniformemente aplicados a la superficie del suelo bajo el árbol para formas una barrera química contra neonatos de *D. abbreviatus*. Populaciones larvales fueron reducidas de 80-100% y estar reducciones persistieron de 4-8 semanas de acuerdo a bioensayos de varias pruebas de campo. En un a casa abierta de tela metálica, bifenthrin dio excelente protección radical a árboles criados en potes durante un periodo de 22 semanas donde neonatos fueron añadidos a los potes semanalmente por 12 semanas. RPA107382 fue altamente efectivo por alrededor de 2 semanas pero careció efecto residual. La acumulación de materia de hojas bajo el árbol perjudico la cobertura del suelo por el químico resultando en control reducido. De acuerdo con cuentas semanales de trampas cebadas, ambos químicos redujeron hormigas forrajeras no-objetivo, particularmente *Solenopsis invicta* Buren. Sin embargo, la reducción de *S. invicta* fue temporera, pero si permitió tiempo para que otras hormigas forrajeras se reestablecieran e incrementaran. Este comportamiento sugiere que control de *S. invicta* puede ser beneficioso.

Diaprepes abbreviatus L., a root weevil native to the Caribbean islands (O'Brien & Wibmer 1984, Woodruff 1985), has gradually become a major localized pest of citrus, many ornamental plants, and some agronomic crops since its introduction into Florida in 1964 (McCoy 1999). It can be a univoltine species on citrus, however, the life cycle can vary greatly in time. The adult, egg, and neonate stage appear on the host plant above ground, and all larval stages, the pupa and teneral adult occur below ground. At hatch, neonates fall from the leaf to the soil surface beneath the tree where they enter the soil (Wolcott 1936). Numerous instars feed on fibrous and woody roots, forming deep grooves in the latter as they consume the outer bark, including the cambium layer. Larvae remain on the roots for 8-15 months, reaching 1.3-2.5 cm in length (Wolcott 1936, Quintela et al. 1998). Injuries caused by *D. abbreviatus* larvae serve as preferred infection courts for root rot diseases of citrus caused by soil-borne fungal pathogens such as *Phytophthora* spp. (Graham et al. 1996). The interaction between root weevils and soil-borne fungal pathogens of the roots results in one of the most severe decline syndromes affecting citrus. Therefore, pest management of *D. abbreviatus* can require treatment of both the insect and soil-borne diseases.

Prior to the cancellation of the chlorinated hydrocarbon insecticides in the U.S.A. around 1980, citrus root weevils were controlled with persistent compounds, such as aldrin and dieldrin (Bullock 1985). These chemicals were generally broadcast on the soil beneath the tree as granules in the dry fertilizer mix. By forming a chemical barrier beneath the tree, invasive neonates were killed before reaching the root system (Bullock 1985). Subsequently, organophosphate and carbamate substitutes for the chlorinated hydrocarbons were found to be less effective because of their shorter residual.

In the past decade, entomopathogenic nematodes, infectious to all soil-inhabiting stages of the weevils, have been applied as biopesticides one or more times per year in Florida citrus (McCoy et al. 2000). Since larval control with nematodes varies in the field and likely occurs in the rhizosphere after larvae are already feeding on the roots (Duncan et al. 1999), various chemical and non-chemical agents for combating neonates at the soil surface have been under investigation. Greenhouse and screenhouse studies using container-grown citrus plants as indicators of neonatal feeding by *D. abbreviatus* have been conducted with systemic and contact pesticides of various formulations. Chlorpyrifos as a slow release granule, imidacloprid and bifenthrin have been shown to be effective against neonates as soil drench or soil-incorporated treatments (McCoy et al. 1995). Bifenthrin is currently recommended for use in citrus nurseries to prevent larval invasion of container-grown plants (Simpson & McCoy 1996).

Bifenthrin is a pyrethroid with a broad-spectrum of activity against insects and mites. In view of its toxicity to neonate root weevils and persistence on the soil surface, screenhouse and field studies were conducted during the past 4 yr with two formulations, Brigade 10WSB® 100% and Capture® 2L, to determine residual control of neonate *D. abbreviatus*. Since bifenthrin is toxic to the imported fire ant, *Solenopsis invicta* Buren, an important predator of *D. abbreviatus* found in citrus groves, ant populations were monitored using baited traps to assess non-target effects.

MATERIALS AND METHODS

Screenhouse Experiment

Sixty-three, 3-yr-old Parson Brown orange trees, grafted to Cleopatra mandarin rootstock, were cleared of soil and pruned slightly for transplant into 56.8 liter plastic containers with a 0.16 m^2 surface area. Each tree was planted in sieved Candler soil (Entisol type: 92% sand, 2.9% clay, 2.0% silt) and placed on a bench in open sunlight. One month after planting, trees were fertilized using liquid 8:4:8 NPK at 60 ml per tree. Trees were watered only when rainfall failed to supply adequate moisture to prevent wilt. Any weeds were periodically removed by hand. Leaf litter, collected from a commercial grove, was scattered on the soil surface to a depth of 1.27 cm of nine trees to compare treatments with and without leaf litter.

Each treatment, Brigade 10WSB at 0.134, 0.269, and 0.54 g/m^2 (high rate equivalent to field rate) and Admire 2L at 0.54 g/m^2, were applied uniformly to the soil beneath the tree in 50 ml of H_2O with a B&G hand held sprayer. Two untreated controls were included in the experiment, one with larval infestation and one without. The treatment with leaf litter received Brigade 10WSB at 0.54 g/m^2. The soil surface of all containerized trees was moistened just prior to treatment and 100 ml of water per tree added immediately after application using a sprinkling can. Each treatment was replicated 9 times. All treatments were applied on May 8, 1996.

At 2 days post-treatment and weekly thereafter, for 12 weeks, 25 neonatal *D. abbreviatus* (48-h-old) were scattered on a moist soil surface of each container. Each container received 325 larvae over time. Neonates used in the study were obtained from field-collected adult females held in screened cages in the greenhouse at 27 ± 2°C.

On October 15, about 22 weeks after treatment, each tree was carefully removed from the container and the soil around the roots washed through a sieve to recover larvae. Soil remaining in the container was also wet-sieved to assure larval recovery. After recording larval number, the root symptoms of each tree were rated visually on a scale from 1 to 5; 1 = no visible injury, 2 = normal fibrous root density, slight tap/lateral root channeling, 3 = moderate fibrous root density and tap/lateral root channeling, 4 = severe fibrous root loss and tap/lateral root channeling, and 5 = no fibrous roots and severe tap/lateral root channeling and stem girdling. In addition, fibrous roots were removed from each tree, dried, and weighed to determine dry root weight. Differences in larval survival/treatment and dry fibrous root weight among different chemical treatments were compared by ANOVA and Tukey's Studentized Range (HSD) test (SAS Institute 1988). Root rates were presented as treatment averages.

Field Test-1

The experiment was conducted in an irrigated 3-yr-old Flame grapefruit grove grafted to 'Swingle' citrumelo rootstock. The grove was located near Alturas, FL on Astatula fine sandy soil. The tree spacing was 3.7 × 6.0 m. Any leaf litter was removed prior to chemical applications with a hand held blower.

Brigade 10WSB was applied at 0.269 and 0.54 g/m^2 (0.276-0.554 kg ai/ha) to a 1.5 m^2 area beneath the tree at 500 ml of finished spray mix at 20 psi using a low pressure sprayer and a hand-held spray wand. Each treatment was comprised of 4-tree plots replicated 10 times and an untreated check. Treatments were applied on April 12, 1996. The grove was irrigated thoroughly before application and for about 1 h after application.

At 0, 31, 59, 95, and 123 days post-treatment, a laboratory bioassay was performed to determine the residual activity of the insecticide in the soil over time. One soil sample of 1.5 ml by volume per tree, 10 per treatment, was taken randomly from the soil surface to a depth of 3.2 mm. Each soil sample was assayed separately by placing each sample in a micro-centrifuge tube (1.5 ml) with 10 neonates. After a 7-day exposure at 27 ± 2°C, larval survival was recorded for each tube. Larval survival representative of each post-treatment bioassay of treated and untreated field soil were made, after the proportions surviving were transformed using arcsine $\sqrt{\chi}$, using a one way ANOVA design followed by Tukey's Studentized Range (HSD) test (SAS Institute 1988). Untransformed means are shown in all figures.

Field Test-2

The experiment was conducted in a 5-yr-old planting of Hamlin orange grafted to 'Swingle' citrumelo rootstock planted in Candler soil, and located on the University of Florida campus, Lake Alfred, FL. The grove was set at a 3.7 × 6.0 m spacing and had micro-sprinkler irrigation. Prior to treatment, leaf litter was removed from the soil beneath the trees. In this test, the residual control of Brigade 10SWS at 0.54 g/m^2 was compared to an untreated check. Plots consisted of 6 trees in one row. Each plot was completely randomized and replicated 4 times. Brigade was applied as a band, 1.8 m in length, to the soil beneath the tree at 369.3 liters/ha using a tractor-mounted herbicide applicator set at 0.2 mpa and traveling at 4.4 kmph. Treatment was applied on September 17, 1998 under clear sky and an air temperature of 26°C. Irrigation was applied for 3 h before application to thoroughly wet the soil and immediately after application for 1 h.

In this test, the bioassay method used to measure residual effect was changed to reflect more typical field conditions. Soil cores were collected randomly from beneath the tree to a depth of 2.54 cm midway between the trunk and dripline using a cork borer with a diameter of 1.27 cm^2. Soil samples were taken weekly, 5 samples per plot or a total of 20 samples per treatment. In the field, each soil core was carefully placed intact, into a plastic column with a screen base (20 mm mesh) just large enough for the passage of larvae through the soil into a well of a plastic tissue culture plate (McCoy et al. 2000). In the laboratory, 10 vigorous neonates (<48-h-old) were placed on the soil surface within the column. After 72 h at 28°C, the number of larvae capable of moving through the soil column into the well of the tissue culture plate was recorded. The number of live, dead, and missing from the original inoculum also was recorded by sorting through the soil. Statistical analysis was performed as described in the previous test.

Field Test-3

The experiment was conducted in a one-yr-old reset planting of Navel orange on 'Swingle' citrumelo rootstock planted in Candler soil and located on the University of Florida campus, Lake Alfred, FL. The grove was close set at a 0.9 × 6.1 m spacing for research purposes and had micro-sprinkler irrigation. Treatments were arranged in a completely randomized block design, each plot consisting of 6 trees/plot (4.6 × 0.5 m). No leaf litter was found on the soil in the experimental site. Treatments consisted of Brigade 10SWS at 0.54 g/m^2 (0.56 kg ai/ha), RPA107382 (0.38 kg EC) at 0.156 and 0.312 ml/m^2 (0.10 kg and 0.19 kg ai/ha) and an untreated check. RPA107382 is an analog of fipronil, a phenyl pyrazole with toxicity to neonatal *D. abbreviatus* (Nigg et al. 1999). Each treatment was replicated 10 times. Chemicals were applied on April 13, 1999 with a backpack CO_2 activated sprayer equipped with a hand held boom with dual fan nozzles. Materials were mixed in 2 liter quantities and applied at 387.2 LPH at 0.2 mpa. At the time of application, air temperature was 18-20°C and relative humidity was 50%. Wind conditions were calm.

At various times, from 0 to 56 days post-treatment, the previously described column bioassay method was used to measure chemical residual on the soil surface. Two soil cores per plot were taken within 15.2 cm of the tree trunk to a depth of 2.54 cm. Bioassay data were analyzed using the procedures described for tests 1 and 2.

Since bifenthrin and fipronil are toxic to *S. invicta* by contact or in bait form (Collins & Callcott 1998, Knapp 2000) and *S. invicta* is a general predator of *D. abbreviatus* (Whitcomb et al. 1982), ant populations were monitored via baited traps pre- and post-treatment to assess non-target effects. About 1.5 g of hamburger was placed on a filter paper strip that was, in turn, placed within a 40 mm plastic assay disk (Millipore) with a small hole on the side to allow for ant entry. A single trap was placed on the soil surface near each tree trunk in each plot. After exposure for 90 min, the trap was closed confining the ants within the disk. In the laboratory, the number and species of ants were recorded. Samples of ants were collected at 1 week pre-treatment and at 7, 14, 21, 29, 35, and 41 days post-treatment. Differences in the total number of ants between treatments was analyzed

TABLE 1. EFFECT OF DIFFERENT RATES OF BRIGADE 10WSB WITH AND WITHOUT LEAF LITTER ON SURVIVAL OF NEONATES OF *DIAPREPES ABBREVIATUS* AND PLANT ROOT HEALTH AFTER WEEKLY HOST INOCULATION FOR 12 WEEKS IN CONTAINER-GROWN CITRUS IN THE SCREENHOUSE.

Treatment	Rate (g/m^2)	Mean number surviving larvae/unit ± SD[a]	Mean fibrous roots, g dry wt/tree ± SD[a]	Mean root[b] rating (1-5)
Control (no larvae)	—	—	1133.9 ± 184 a	1.0
Control (larvae)	—	15.1 ± 9.0 bc	589.7 ± 102 c	4.2
Brigade 10WSB	0.143	8.3 ± 6.7 ab	816.5 ± 197 c	2.5
Brigade	0.269	22.8 ± 9.4 c	861.8 ± 391 bc	2.7
Brigade (no litter)	0.54	8.8 ± 4.1 ab	1179.3 ± 304 a	1.7
Bridge (litter)	0.54	23.8 ± 11.4 c	771.1 ± 164 c	3.3
Admire 2F (no litter)	0.54	6.0 ± 3.6 ab	997.9 ± 201 ab	1.3

[a]Means followed by the same letter are not significantly different at the 5% level of probability using Tukey's Studentized Range (HSD) test.
[b]Root symptoms: 1-no visible injury, 2-slight tap/lateral root channeling, 3-moderate tap/lateral root channeling, 4-severe tap/lateral root channeling, 5-severe tap/lateral root channeling and stem girdling.

statistically for each sample date via ANOVA and Tukey's Studentized Range (HSD) test.

RESULTS

Screenhouse Experiment

As expected, the control with neonates had greater root injury than the control with no larvae (Table 1). Fibrous root loss in the larval control was nearly twice that of the plant only control and overall root injury was severe. Only 5% of the original larvae introduced were recovered at the end of the test.

The presence of leaf litter on the soil surface of the containerized citrus trees reduced larval control by Brigade suggesting that the soil barrier was distorted by litter. As shown in Table l, Brigade at 0.54 g/m^2, without leaf litter, resulted in higher larval mortality and root protection com-

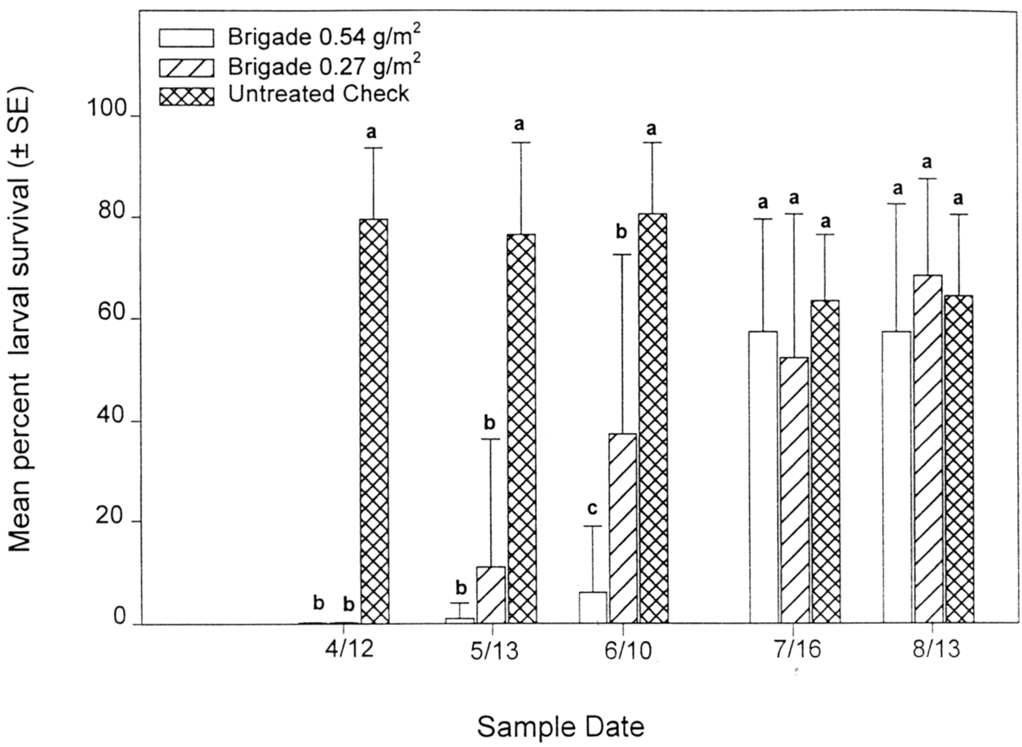

Fig. 1. Residual effect of two rates of Brigade 10SWS on neonate survival of *Diaprepes abbreviatus* based on surface soil sampling at Alturas, FL—"bars with common letters on the same sampling date are not significantly different" at the P = 0.05 level.

pared to the treatment with leaf litter; in fact, there was no significant difference in larval survival or root protection between Brigade with litter, the control without larvae, and the Admire standard. As the rate of Brigade decreased, root protection also decreased (Table 1). In view of its systemic action, Admire as a standard without litter performed as well as Brigade without litter and the control without larvae.

Field Test-1

As shown in Fig. 1, the residual control of Brigade against neonate *D. abbreviatus*, based on surface soil bioassays, was significantly longer (>59 days) at the highest rate (0.54 g/m^2) than at the lower rate (0.269 g/m^2); but, the lower rate was significantly different from the untreated check (P = 0.05). At 95 days post-treatment or longer, all treatments were the same and provided no control.

Field Test-2

In the fall study, the previously described soil column bioassay method, more typical of what invasive larvae would experience in the field, was used to monitor residual control of the chemical barrier to neonate *Diaprepes*. As shown in Fig. 2, Brigade at 0.54 g/m^2 gave 100% kill of neonates in a bioassay performed within 72 h after treatment. At 1 week post-treatment, larval survival increased to about 20% but remained significantly lower than the untreated check. Residual control varied from 40-58% compared to the untreated check during the 8-10 weeks after treatment. Significant difference between treatments was measured via bioassay for about 3 months; however, larval survival approached 80% by that time (Fig. 2).

Field Test-3

In the spring study, using the soil column bioassay, both Brigade at 0.54 g/m^2 and the 2 rates of RPA107382 at 0.156 and 0.312 ml/m^2 significantly reduced larval survival compared to the control at day 1 post-treatment (Fig. 3). In the case of Brigade, residual effect resulted in less than 30% larval survival throughout the study (8 weeks), whereas, the residual effect of RPA107382 began to fail at 4 weeks post-treatment and was less effective than Brigade thereafter, but significantly better than the control through 6 weeks post-treatment.

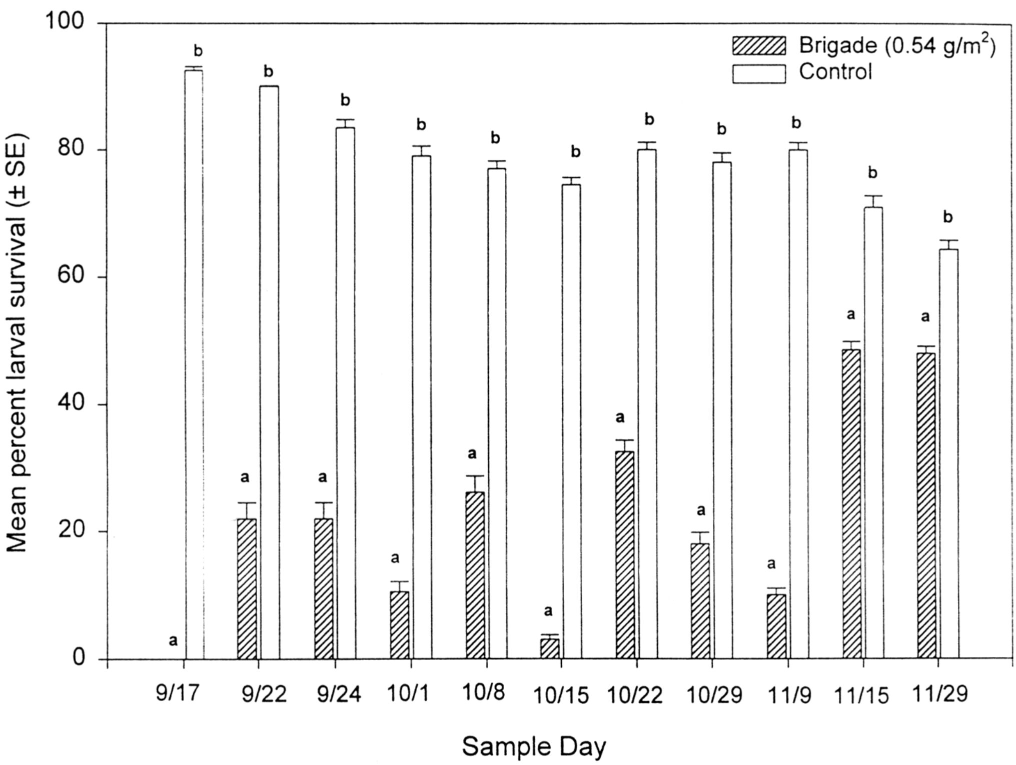

Fig. 2. Residual effect of Brigade 10SWS applied in the fall on neonate survival of *Diaprepes abbreviatus* based on soil column bioassay at Lake Alfred, FL—"bars with common letters on the same sampling date are not significantly different" at the P = 0.05 level.

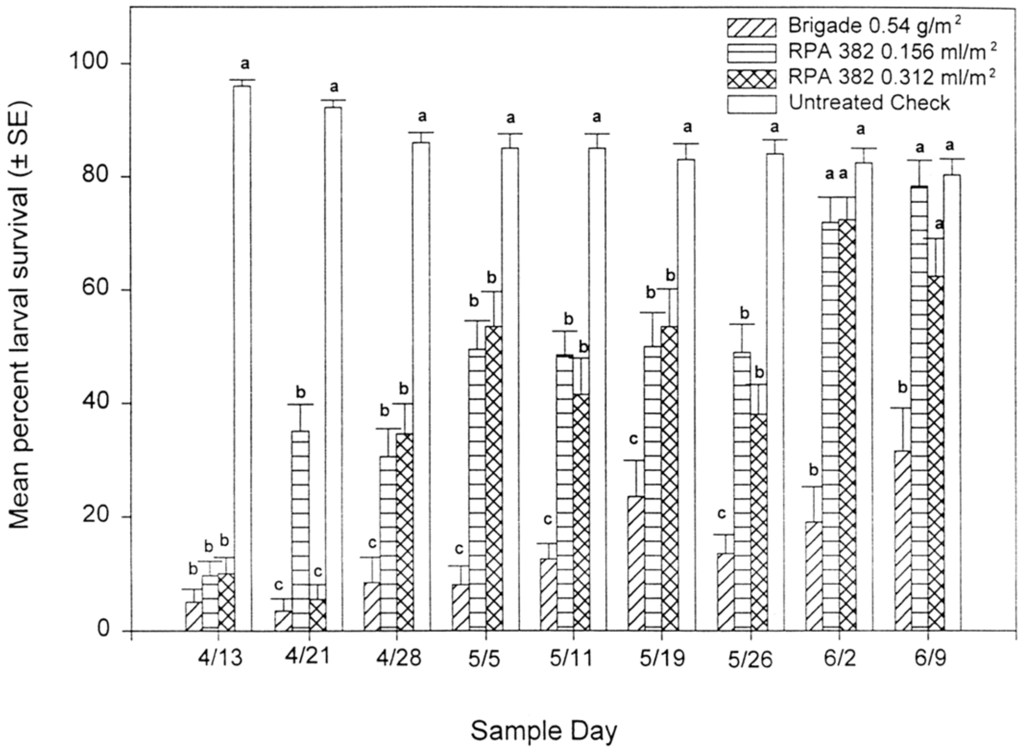

Fig. 3. Residual effect of Brigade 10SWS and two rates of RPA107382 on neonate survival of *Diaprepes abbreviatus* based on soil column bioassay at Lake Alfred, FL—"bars with common letters on the same sampling date are not significantly different" at the P = 0.05 level.

The effect of the different chemicals applied to the soil on non-target foraging ant populations over time is depicted in Fig. 4 and Table 2. In the control, *Solenopsis invicta* Buren was always most frequently trapped using hamburger bait followed by *Dorymyrmex bureni* (Trager), *D. reginicula* (Trager), *Brachymyrmex obscurior* Forel, and *Tetramorium simillimum* (F. Smith) in descending order of abundance. As shown in Fig. 4, both Brigade and RPA107382 suppressed *S. invicta*, at a very low level, through 29 days post-treatment. Thereafter, *S. invicta* gradually increased in abundance. Other foraging ants mentioned above were not eliminated and after 29 days both *S. invicta* and other ants increased together at 35 and 41 days post-treatment (Fig. 4). As shown in Table 2, *D. bureni* appeared to become the prevalent foraging ant following the chemical treatments.

Discussion

Screenhouse and field data collected at different times of the year for 4 yr using two bioassay methods all support the efficacy of bifenthrin (Brigade/Capture) as a chemical barrier against neonate *D. abbreviatus*, if leaf litter does not interfere with coverage of the soil. Since bifenthrin has the propensity to bind strongly to soil particles after drying, uniform application to a moistened substrate free of debris appears vital for maximum contact with the invasive larvae. Since young trees (<5 yr old) do not accumulate leaf litter in large quantity, a chemical barrier should be effective. Timmer et al. (2001) showed that leaf litter accumulation beneath mature trees is greatest from January through June. Unfortunately, this coincides with the spring adult emergence of *D. abbreviatus.* Removal of leaf litter by air blast using a speed sprayer appears to be a feasible way to redistribute the litter away from the tree.

According to bioassays, the most effective rate of bifenthrin to reduce neonate populations entering the soil appears to be 0.54 g/m^2 (0.554 kg ai/ha). A rate of 0.269 g/m^2 will reduce neonates within 7 days after soil application, however, residual effect is lesser in the field. Effect of bifenthrin on other instars and teneral adults is unknown. Obviously, the adult stage would be exposed to the chemical barrier at the time of emergence.

Although bifenthrin and RPA107382 reduced *S. invicta* populations following soil application, this reduction appeared to favor the re-establishment of more diversity at baits in the ant fauna among the treatments (Fig. 4). Data suggest that this faunal shift at baits occurred following a significant reduction of *S. invicta*. In view of its dom-

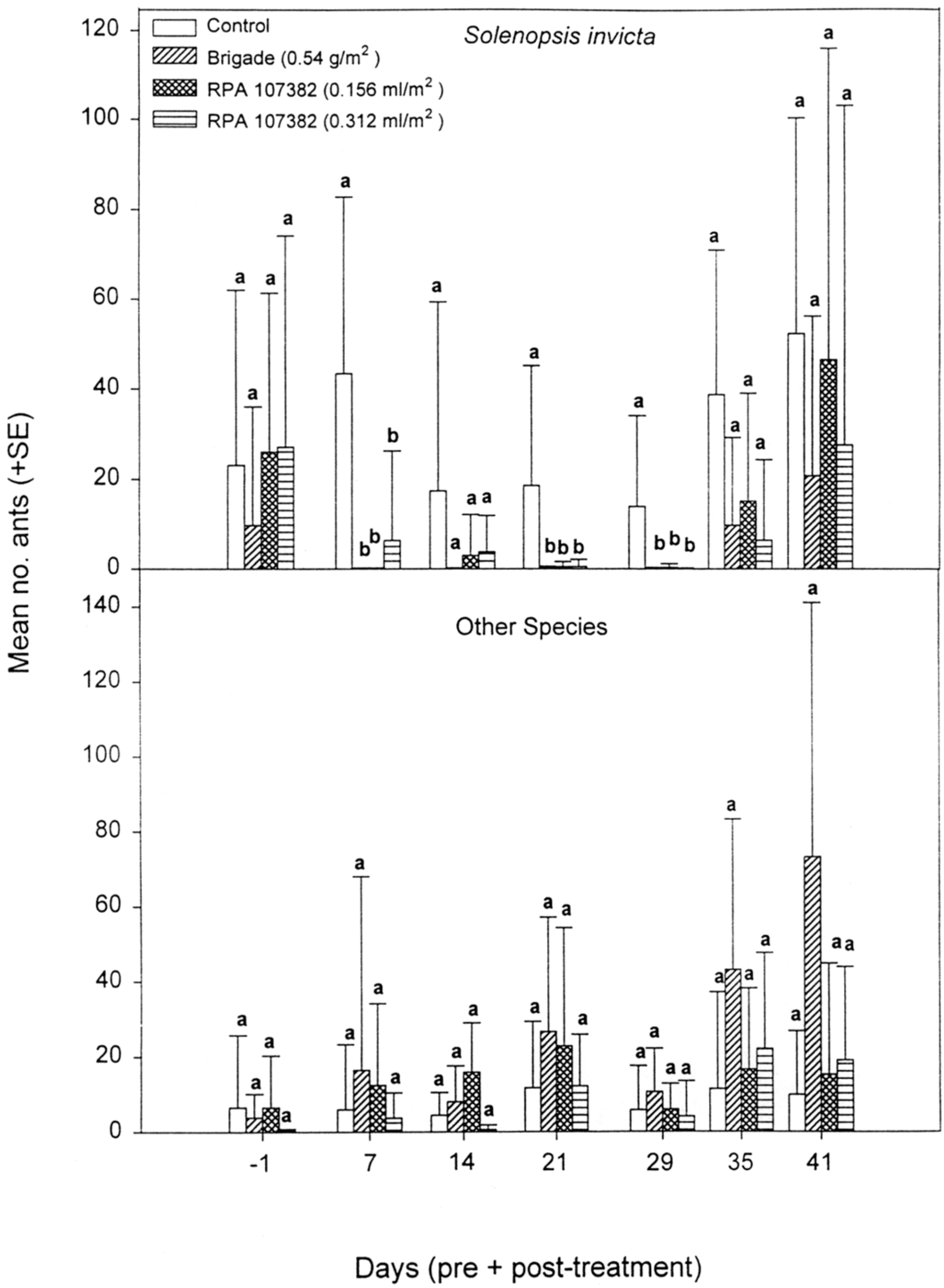

Fig. 4. Mean number of *Solenopsis invicta* and other foraging species trapped over time on the soil surface via baited traps following the field application of Brigade and RPA107382 for root weevil larval control, Lake Alfred, FL. Application made at zero days—"bars with common letters on the same sampling date are not significantly different" at the P = 0.05 level.

inance in disturbed habitats such as citrus groves (Tschinkel 1988), its reduction could have caused the shift and therefore, might be beneficial, if the re-establishment of a more diverse ant community improves predation on neonates. These data and other unpublished work show that *S. invicta*

TABLE 2. TOTAL PERCENTAGE OF DIFFERENT ANT SPECIES TRAPPED ON THE SOIL SURFACE VIA BAITED TRAPS AFTER 41 DAYS FOLLOWING FIELD APPLICATION OF SOIL INSECTICIDES, LAKE ALFRED, FL.

Treatment	Species	%
Control	*Solenopsis invicta*	74.8
	Dorymyrmex bureni	10.2
	Dorymyrmex reginicula	10.3
	Brachymyrmex obscurior	0.3
	Tetramorium simillimum	4.4
Brigade 10SWS (0.54 g/m^2)	*Solenopsis invicta*	18.1
	Dorymyrmex bureni	69.7
	Brachymyrmex obscurior	3.8
	Tetramorium bicarinatium	8.4
RPA 107382 (0.156 ml/m^2)	*Solenopsis invicta*	48.8
	Dorymyrmex bureni	43.8
	Brachymyrmex obscurior	2.7
	Tetramorium simillimum	4.7
RPA 107382 (312 ml/m^2)	*Solenopsis invicta*	53.6
	Dorymyrmex bureni	41.4
	Brachymyrmex obscurior	1.1
	Tetramorium bicarinatium	3.9

will recover however, and re-establish its dominance at baits within a few weeks, suggesting that periodic treatment for *S. invicta* could be a positive management practice. Further research is appropriate.

Hamburger was a successful bait for capturing *S. invicta* and other foraging ants. It should be pointed out however, that some selection of ant species likely occurred because of a preference for meat and aggressive dominance at baits by particular species.

Seasonal population dynamics of adult *Diaprepes* based on trapping data suggest that peak emergence occurs in April through mid-June in irrigated citrus groves in central Florida with some emergence throughout the year (Stansly et al. 1997, McCoy & Duncan 2000). Since adults are most abundant at this time of the year, one can assume that oviposition and subsequent neonatal invasion of the soil is also at a high level, particularly in view of the fact that ovipositing adults apparently live 3-4 months in the field and food is abundant. According to this biological information, it would appear that the use of bifenthrin should begin in late April to early May to maximize its residual effect on invasive larvae. Studies are currently underway to evaluate this strategy over time.

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