Response of Argentine Ants and Red Imported Fire Ants to Permethrin-Impregnated Plastic Strips: Foraging Rates, Colonization of Potted Soil, and Differential Mortality

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ABSTRACT This study investigated the effects of the permethrin-impregnated plastic on ant mortality and foraging rates, and tested its potential for preventing ants from colonizing potted soil. Direct exposure to the plastic for as short as 1 min caused significant mortality of both red imported fire ants, *Solenopsis invicta* Buren, and Argentine ants, *Linepithema humile* (Mayr); however, red imported fire ants were more susceptible than Argentine ants. Knockdown of virtually all ants initially occurred within 15 min after exposure. However, some moribund ants recovered from the effects within 24 h. For example, after 1 min of direct exposure to the permethrin-impregnated plastic, 70% of Argentine ants and 5% of red imported fire ants recovered from the treatment. In established colonies of Argentine ants, significantly fewer ants foraged for food up posts treated with the plastic compared with untreated posts. In addition, colonies responded to introduction of the treatment by significantly reducing their overall foraging rates, even on untreated posts. When pots filled with moistened soil were introduced into established ant colonies, 82% of Argentine ants and 99% of red imported fire ants moved into the soil. In contrast, when a 1-cm-wide coil of the plastic was placed under the pot, no ants moved into the soil. The potential for use of these materials in nursery production is discussed.

KEY WORDS *Linepithema humile*, *Solenopsis invicta*, insecticide, permethrin-impregnated plastic

ANT INFESTATIONS ARE A widespread problem for the nursery industry. Some species, such as Argentine ants, *Linepithema humile* (Mayr), tend colonies of hemipteran pests such as aphids, scales, and whiteflies. The ants collect their honeydew as a food source and actively interfere with natural enemy activity against these pests, resulting in larger populations when ants are present (DeBach 1958, Addicott 1979, Bristow 1984). Species such as Argentine ants; southern fire ants, *Solenopsis xyloni* McCook; and red imported fire ants, *Solenopsis invicta* Buren, will readily nest in the pots. Infestations of container plants with any ant species can result in delay or rejection of inter-regional shipments because of quarantines presently in place for *Solenopsis* spp. and other pests. For example, Lewis et al. (1992) reported that nursery stock accounted for 5% of the interceptions of red imported fire ant at border stations in California, and commercial shipments of plants contributed to the widespread distribution of Argentine ants in the United States (Newell and Barber 1913, Smith 1965, Vega and Rust 2001).

California is the leading producer of nursery products in the United States. In 2002, nursery products ranked second in the state among agricultural commodities produced with a value of $2.4 billion (CASS 2003). The recent introduction of the red imported fire ant into southern California has drastically increased the use of pesticides for ant control in nurseries. Under current red imported fire ant quarantine procedures, an entire nursery must be treated if even a single fire ant is found. Treatments include sprays containing chlorpyrifos (organophosphate), diazinon (organophosphate), and bifenthrin (pyrethroid), and baits containing hydramethylnon, and fenoxycarb (insect growth regulator). The mandated use of these materials resulted in a dramatic increase in the amount of insecticide applied. For example in Orange County, bifenthrin use in nurseries increased from ≈50 lb (AI) applied in 1997 to >600 lb (AI) applied in 1999 (CDPR 1997, 1999). It is estimated that nurseries in California currently spend $700–1000 per acre to treat for red imported fire ants (Klotz et al. 2003, Kabashima 2005).

In addition to insecticide costs, there are obvious disadvantages associated with these treatment practices. Applicators must wear protective clothing during insecticide applications. Chlorpyrifos and bifenthrin have been detected in nursery water runoff, pose a hazard to fresh water organisms, and also are implicated in legal issues pertaining to the potential polluted runoff entering nearby urban streams and eventually large creeks or ocean estuaries (Walters et al. 2002, Kabashima et al. 2003, Bondarenko et al. 2004). Alternative methods to prevent ants from establishing colonies in nursery stock are needed to reduce the amount of insecticides being used and their potential impact in the environment. The objectives of the project are to determine the effects of a permethrin-impregnated plastic on ant mortality and foraging rates and to investigate its potential as an alternative method for preventing ants from colonizing potted soil.

Materials and Methods

Timed Forced Exposure. The insecticide-impregnated material used in this study (ARINIX, Nix of America, San Jose, CA) was composed of a plastic impregnated with 9% permethrin. The mortality rates of Argentine and red imported fire ants were compared after direct forced exposure to this material in small glass vials. Argentine ants were collected from field populations in Riverside, CA; red imported fire ants were collected from field populations in Lake Elsinore, CA. Groups of 10 ants were exposed to a 1 cm^2 × 2-mm-thick piece of permethrin-impregnated plastic secured to the bottom of a glass vial (1 by 4 cm) or to a control vial without plastic. The walls of the vial were coated with Teflon (fluropolymer resin, type 30, DuPont Polymers, Wilmington, DE) to prevent ants from escaping, and forcing them to remain at the bottom of the vial on the surface of the plastic. Ants were exposed for 0.5 (red imported fire ants only), 1, 3, 5, or 10 min (Argentine ants only) at room temperature (24 ± 1°C). After each exposure, vials were inverted and tapped to empty ants into clean petri dishes supplied with water and held at room temperature. The number of ants alive in each dish was counted at 0.25, 0.5, 1.5, 3, 5, 24, and 48 h after exposure. Four replications for each exposure period were completed.

For each species, ant mortality in treated and control groups was statistically compared at each observation time after exposure using Kruskal–Wallis one-way analysis of variance (ANOVA), pooling results for all exposure periods. The relative susceptibility of Argentine ants versus red imported fire ants was compared by a univariate repeated measures ANOVA, which compares the two species using the means over time of the 1-, 3-, and 5-min exposure periods (Systat 9, SPSS Inc. 1999).

Effects on Foraging Rates of Argentine Ants. Six colonies of Argentine ants collected from field populations in Riverside were set up in plastic containers (25 by 30 by 10 cm) in the laboratory. Each consisted of 2,000–3,000 ants, including two or more queens, and some larvae. The walls of the containers were coated with Teflon to prevent ants from escaping. Each colony was provided with an artificial nest constructed from a plaster-filled petri dish (10 cm in diameter by 1 cm in depth) formed with a 4.5-cm-diameter by 0.5-cm deep circular area in the center of the dish to serve as an artificial nest referred to as ant condos. Dishes were covered with a lightproof disk that could be removed to observe the number of ants and queens in a nest. Moisture was applied to nests through wicks embedded in the plaster. Two polyvinyl chloride (PVC) posts (1 cm in diameter by 20 cm in height) were placed in each colony in a vertical position. A 10-cm-diameter petri dish lid was attached horizontally to the top of each post and provisioned with two weigh boats (3.5 cm in diameter by 1 cm in depth), one containing 25% sugar water and the other plain water (Fig. 1). Studies were conducted in a walk-in growth chamber at 27°C. Colonies were allowed to acclimate for 5 d, and ants were actively foraging at every feeding station. Initial foraging rates were established by averaging the number of ants climbing up each post in four 2-min intervals.

Fig. 1. Colonies of Argentine ants reared in plastic boxes. Each colony was provided with an artificial nest (condo). Two PVC posts were placed in each colony with a petri dish lid attached horizontally to the top of each post and provisioned with two weigh boats, one containing 25% sugar water and the other plain water.

Three of the colonies were randomly selected to receive a permethrin-impregnated plastic treatment. In these colonies, a small section of plastic coil (1 cm in diameter) was wrapped around one of the two PVC posts (randomly selected) covering a section of the post such that ants had to walk over a 2-cm-long treated section to reach the feeding station. Masking tape was used to cover a similar-sized section on the remaining post in these three colonies. Thus, ants in each treated box had a choice of foraging up a treated or an untreated post. No insecticide treatment was added to the other three colonies; masking tape was used on both posts in those boxes. Foraging rates of ants climbing each post in all containers were counted at 0.25, 3, 24, 48 and 192 h after treatment was applied.

Foraging rates on treated and untreated posts within treated boxes were compared at each observation time using a paired sample *t*-test. Overall foraging rates in treated and untreated boxes were compared by ANOVA on the means of the two groups over time (Systat 9, SPSS Inc. 1999). At the end of the study, the total number of dead ants was counted, and the mean number of dead ants in treated and untreated boxes was compared using a *t*-test.

Effect on Colonization of Potted Soil by Argentine Ants and Red Imported Fire Ants. A molded plastic coil of the permethrin-impregnated plastic (1 cm in width by 24 cm in length) was formed into a circular ring (9 cm in diameter) and glued to the base of three nursery pots (10 by 10 by 9 cm in height) filled with moist soil, such that the ants had to cross the plastic barrier to enter the pot. A similar-sized circular ring of plastic poly tubing was glued to the base of three additional pots to be used as controls.

Six colonies of Argentine ants and six colonies of red imported fire ants were assembled and maintained in plastic containers as described above. Argentine ants were collected from field populations in Riverside; 300–500 worker ants were introduced into each container to establish colonies. Red imported fire ants were collected from field populations in Lake Elsinore; 1,500–2,000 worker ants were introduced into each container. In addition to an artificial nest (described above), each colony container also was provided with one water supply wick and a small dish of 25% sucrose solution.

After ants had acclimated for 1 wk, either one treated or one untreated pot of moistened soil was introduced into each colony. The experiments with Argentine ants were conducted at the University of California Campus, Riverside, in a walk-in growth chamber maintained at 27°C. Experiments with red imported fire ants were conducted at the University of California South Coast Research Station in Irvine, CA, in a laboratory kept at room temperature (27°C). Treatments were arranged in a completely randomized design; ant colonies were randomly selected to receive treated or untreated pots. At 1 and 7 d after introduction, pots were observed for the presence of ants moving in or on the soil. The number of ants present in the soil and in the artificial nest was counted 7 d after the introduction of the pots. The 95% CI was calculated for the mean number of ants present in the soil of treated and untreated pots (Systat 9, SPSS Inc. 1999).

Results

Timed Forced Exposure. Forced exposure to the permethrin-impregnated plastic caused significant mortality for both species of ants at each observation time after treatment (Fig. 2) (Kruskal-Wallis one-way ANOVA, $N = 32$, $P \ll 0.001$ for all times for both species). Even the shortest exposure periods tested resulted in significant mortality compared with controls (Argentine ant: $F = 139$; df = 1, 6; $P \ll 0.001$; red imported fire ant: $F = 5276$; df = 1, 6; $P \ll 0.001$).

For both ant species, and for all exposure periods, all ants seemed moribund at 15 min after direct exposure to plastic. However, after the initial knockdown, some ants began to recover from the exposure (Fig. 2). For example, with Argentine ants that were exposed to the plastic for 1 min, some individuals began to recover from the treatment within 3 h after exposure, and >70% of ants recovered within 24 h. When the exposure period was increased to 3 min, fewer ants recovered (28%) overall. Increasing the exposure period to five or 10 min did not increase mortality rate, with 30–35% of ants recovering within 24 h. Red imported fire ants were more susceptible to the impregnated plastic than were Argentine ants (univariate repeated measures ANOVA of the two species over time [comparing 1-, 3-, and 5-min exposure periods only]; $F = 18.45$; df = 1, 22; $P < 0.001$). Only 2–5% of red imported fire ants recovered from direct exposure, even if only exposed for a period of 0.5 min (Fig. 2).

Effects on Foraging Rates of Argentine Ants. Within the boxes treated with permethrin-impregnated plastic, when ants were given a choice to forage for food on top of treated or untreated posts, significantly fewer ants were observed foraging up the treated posts than untreated posts at each time recorded up to 8 d after treatment (Table 1).

Although the treatment was applied to only one post within each box, the total foraging rate at each observation time after treatment application was significantly lower than the pretreatment foraging rates, indicating that the presence of the treatment reduced foraging up the treated and untreated posts. When the total foraging rate in treated boxes was compared with the untreated boxes, there were significantly fewer ants foraging in boxes with treated posts ($F = 40.32$; df = 1, 46; $P \ll 0.001$; Fig. 3). The total number of ants foraging in treated boxes dropped within 15 min after the treatment was applied and remained lower than untreated boxes at each time interval until the end of the experiment (8 d) (Fig. 3). Foraging rates

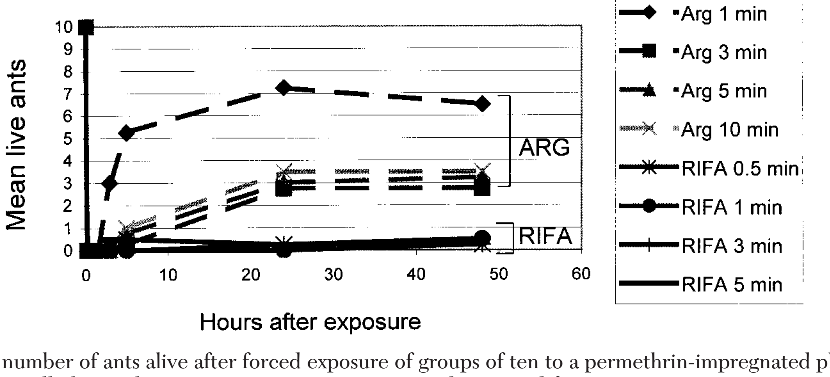

Fig. 2. Mean number of ants alive after forced exposure of groups of ten to a permethrin-impregnated plastic for 0.5, 1, 3, 5, or 10 min in small glass vials. ARG, Argentine ants; RIFA, red imported fire ants.

Table 1. Pairwise comparison (paired sample *t*-test) of mean no. (±SE of means) of ants foraging up a permethrin-impregnated plastic-treated or an untreated post within the same colony during a 2-min observation period

	Pretreatment	Hours after treatment				
		0.25	3	24	48	192
Treated post	8.17 ± 3.61	0 ± 0	0 ± 0	0 ± 0	0.08 ± 0.08	0.25 ± 0.14
Untreated post	8.92 ± 2.82	1.92 ± 0.50	3.83 ± 0.82	1.33 ± 0.58	1.50 ± 0.90	2.67 ± 0.73
t-value	0.664	3.727	5.702	3.218	3.02678	4.69880
df	11	11	11	11	11	11
P value	0.52017 NS	0.00334	0.00014	0.00819	0.01152	0.00065

P values < 0.05 are significant; NS, not significant.

in untreated boxes remained relatively constant (Fig. 3).

Mean mortality in treated (mean number of ants ± SEM, 30 ± 6) and untreated (48 ± 21) boxes was not significantly different ($t = 1.625$, df = 8, $P = 0.14252$), indicating that the reduction in ant foraging rate was because of behavioral changes and not increased mortality as a result of treatment. Ants within the colony responded to the treatment by significantly reducing their foraging rates even on untreated posts.

Effect on Colonization of Potted Soil by Argentine Ants and Red Imported Fire Ants. After untreated pots filled with moistened soil were introduced into established ant colonies, the majority of the individuals within colonies (82% of Argentine and 99% of red imported fire ant colonies) moved out of their established ant condos into the untreated pots. In contrast, when a coil of permethrin-impregnated plastic was attached to the base of the pots, no ants colonized soil in the pots. For Argentine ants, 214 ± 82 (mean number of ants ± 95% CI) moved into untreated pots and zero moved into treated pots. For red imported fire ants, 1,954 ± 83 ants moved into untreated pots, and zero moved into treated pots. A single 1-cm ring of the plastic was successful in preventing both red imported fire ants and Argentine ants from infesting the potted soil.

Discussion

Overall, the permethrin-impregnated material tested was effective against both Argentine ants and red imported fire ants and caused significant mortality after contact, reduced foraging rates over treated areas, and prevented colonization of moist soil. The significantly greater survival of Argentine ants compared with red imported fire ants in the forced exposure experiment was unexpected. Given the larger average size of red imported fire ant workers, one might expect them to have greater survival. For example, in a vineyard study, Addison (2002) found it was easier to control Argentine ants with stem sprays and bands than to control the larger *Anoplolepis* spp. She speculated that the larger size and longer legs of *Anoplolepis* resulted in less contact with the treated surface.

The behavioral response of ants after the introduction of the treatment to one of two feeding sites in the colony is noteworthy. The observation that significantly fewer ants foraged up treated posts compared with untreated posts demonstrates that the toxic material was having a negative impact on foraging. Deposits of permethrin were completely repellant to Argentine ants within 60 min after being exposed (Knight and Rust 1990). Compared with other pyre-

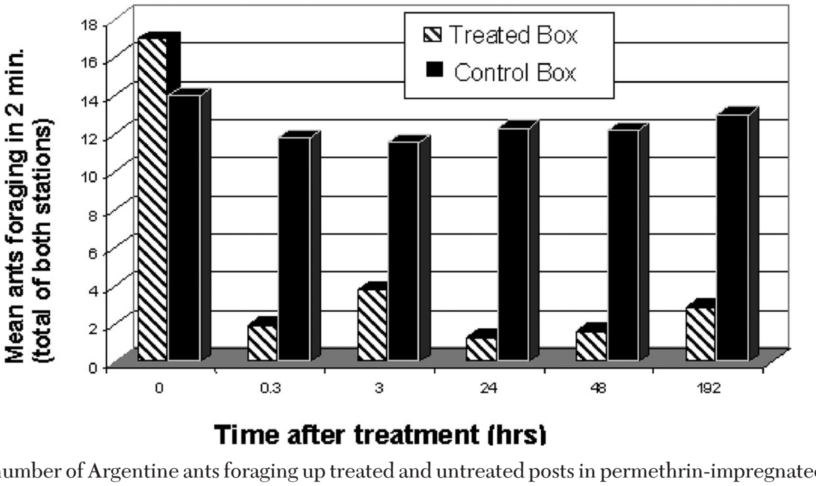

Fig. 3. Total number of Argentine ants foraging up treated and untreated posts in permethrin-impregnated plastic-treated or untreated boxes.

throids and organophosphates, permethrin was considered to be moderately repellant and toxic to *L. humile.*

A study by Helmy and Jander (2003) demonstrated that black carpenter ants, *Camponotus pennsylvanicus* (De Geer), on their way to and from a food source, learned to associate a chemical stimulus with the decision to climb a specific vertical dowel to find food. Thus, it is possible that Argentine ants learned to associate a chemical stimulus with a decision *not* to forage for food in a particular area. Such a stimulus could be the insecticide itself or a signal or marker produced by ants to warn others to avoid foraging in the treated area.

The observation that foraging rates at untreated food sources also were dramatically reduced after treatment might be the result of horizontal transfer of the insecticide causing sublethal effects and inhibiting foraging. Mortality of Argentine ants resulting from horizontal transfer of insecticides such as fipronil to untreated individuals has been reported previously (Soeprono and Rust 2004). However, in our study, there was no significant difference in mortality rates between treated and untreated colonies, suggesting a behavioral response to a sublethal dose. When the effects of the material on the colony were realized, ants may have responded not only by specifically avoiding the site of toxicity but also by avoiding foraging in general. A similar response was observed with Argentine ant colonies after exposure to potted soil treated with fipronil (Costa and Rust 1999).

Various strategies to exclude ants from foraging or nesting in a specific area have been tried in the past. In the early 1900s, for example, citrus growers in Louisiana created water-filled ditches around groves to prevent the spread of Argentine ants (Newell and Barber 1913), and they excluded them from trees by banding with a mixture of a sticky substance (unspecified type of thinner) plus carbolized oil or sulfur (Barber 1916, 1920). For indoor use, a bichloride-of-mercury tape was recommended that was wrapped around furniture legs to keep Argentine ants off the furniture (Barber 1916). More recently, Shorey et al. (1992, 1993, 1996) formulated mixtures of the polybutene preparation Stickem Special (Seabright, Emeryville, CA) with repellents to Argentine ants and applied them to cotton twine, for wrapping around tree trunks. They found the synthetic chemical farnesol to be the most effective repellent, excluding ants from foraging in the canopy for 5 mo (Klotz et al. 1997). Controlled release chlorpyrifos bands provided even longer term control, excluding ants from citrus trees for up to 229 wk (James et al. 1998). Soft plastic strips impregnated with permethrin (Line Guard Inc., Elyria, OH) have been successfully used to band tree trunks to deter Argentine ants for ≈7 mo (Flint and Dreistadt 2004). However, materials such as these have not been tested on red imported fire ants or for their ability to stop ants from infesting potted soil in nursery conditions.

Despite the potential efficacy of banding to exclude ants, growers have not adopted this technique because repeated replacement of the bands is labor-intensive and not cost-effective (Vega and Rust 2001). Materials tested previously did not last long enough to protect nursery crops that are grown for one or more years without replacement. The permethrin-impregnated material tested in this study was originally designed for use as a molded component in automobiles to deter spiders from entering the vent orifice of the fuel system and was designed to last for 10 yr (Nix of America). In laboratory studies against red imported fire ants, this material showed efficacy toward ants after being aged for 5 yr (Drees et al. 2005). Because the product is made of a molded plastic material, it can be designed to accommodate any shape required to its application. Such materials have potential for use in preventing soil infestation of ants in container nurseries if the material can be economically incorporated as a portion of a molded pot, as a collar or coaster attached to pots that could be removed before shipping and reused, or if incorporated into woven mat material commonly used to control weeds in the nursery. The amount of insecticide that would be needed for ant exclusion if the strips were strategically placed would be negligible and carry minimal risk to the environment because the active ingredient is bound to the plastic.

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