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Source: Journal of Economic Entomology, 103(5):1761-1769. 2010.
Published By: Entomological Society of America
DOI: 10.1603/EC10154
URL: http://www.bioone.org/doi/full/10.1603/EC10154

Development of Virtual Bait Stations to Control Argentine Ants (Hymenoptera: Formicidae) in Environmentally Sensitive Habitats

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J. Econ. Entomol. 103(5): 1761–1769 (2010); DOI: 10.1603/EC10154

ABSTRACT A novel bait station referred to as a virtual bait station was developed and tested against field populations of the invasive Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae), at White Beach, Camp Pendleton, in Oceanside, CA. White Beach is a nesting habitat for an endangered seabird, the California least tern (*Sterna antillarum browni* Mearns). The beach is heavily infested with Argentine ants, one of the threats for the California least tern chicks. Conventional pest control strategies are prohibited because of the existence of the protected bird species and the site's proximity to the ocean. The bait station consisted of a polyvinyl chloride pipe that was treated on the inside with fipronil insecticide at low concentrations to obtain delayed toxicity against ants. The pipe was provisioned with an inverted bottle of 25% sucrose solution, then capped, and buried in the sand. Foraging ants crossed the treated surface to consume the sucrose solution. The delayed toxicity of fipronil deposits allowed the ants to continue foraging on the sucrose solution and to interact with their nestmates, killing them within 3–5 d after exposure. Further modification of the bait station design minimized the accumulation of dead ants in the sucrose solution, significantly improving the longevity and efficacy of the bait station. The virtual bait station exploits the foraging behavior of the ants and provides a low impact approach to control ants in environmentally sensitive habitats. It excluded all insects except ants, required only milligram quantities of toxicant, and eliminated the problem of formulating toxicants into aqueous sugar baits.

KEY WORDS *Linepithema humile*, fipronil, horizontal transfer, California least tern

Several species of ants, such as the Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae); red imported fire ant, *Solenopsis invicta* Buren; Pharaoh ant, *Monomorium pharaonis* (L.); and southern fire ant, *Solenopsis xyloni* McCook, present a serious problem to humans, domestic pets, and wildlife (Klotz et al. 2008a). When sensitive sites (e.g., hospitals, nursing homes, parks, and schools) or environments (e.g., islands, estuaries, coastal ecosystems, and endangered wildlife preserves) are infested with these ants, control options are often limited because of potential exposure of humans, nontarget organisms, or both to insecticides or the contamination of sensitive ecosystems. In these situations, baiting is the most environmentally friendly approach for control because the insecticide is contained in the bait stations so that there is minimal exposure of nontarget organisms and the environment (Hooper et al. 1998, Klotz et al. 2004, Klotz et al. 2008a, Gentz 2009).

Ant baits consist of an active ingredient mixed with food or other attractive substances. Because different species of ants prefer different food substances, the acceptance, transport, and transfer of active ingredients throughout the colony—and their resulting efficacy—are significantly affected by their feeding preference (Klotz et al. 2008a). Insecticide-laced solid protein baits have been extremely effective against the Pharaoh ant (Oi et al. 1994) and southern fire ant (Hooper et al. 1998). Solid baits incorporating slow-acting toxicants such as hydramethylnon, insect growth regulators, and indoxacarb formulated in soybean oil have been effective against many myrmicine ants such as the red imported fire ant (Oi and Oi 2006), Pharaoh ant (Oi et al. 1994), and harvester ants (Wagner 1983). However, many species of dolichoderine ants do not readily recruit to and forage on these oil-based baits. For example, these oily baits are less effective against the Argentine ant and odorous house ant, *Tapinoma sessile* (Say), which prefer aqueous sugar solutions instead (Wagner 1983, Rust and Knight 1990, Klotz et al. 2000). However, formulating toxicants in an aqueous sugar solution presents a special problem because most potentially active toxicants are not very soluble in water (Rust et al. 2004).

Horizontal transfer of insecticides is the movement of an active ingredient from one individual to another individual (Buczkowski and Schal 2001, Soeprono and

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Rust 2004). In ants, the horizontal transfer of active ingredients typically occurs if ants ingest and store a liquid toxic bait in their crop and then regurgitate it to feed other nestmates via trophallaxis. However, some insecticides transfer horizontally from one ant to another by physical contact without any food exchange. For example, when worker ants were exposed to sand substrates treated with fipronil and returned to small laboratory colonies, fipronil was horizontally transferred to nestmates and killed the recipients (Soeprono and Rust 2004). Grooming and necrophoresis (removal of dead nestmates) were the primary modes of insecticide transfer (Choe and Rust 2008). Many of the insecticides commonly applied as chemical barriers by pest management professionals have been tested for their ability to be transferred from one ant to another. Only fipronil and possibly high concentrations of thiamethoxam provided horizontal transfer and kill of workers (Choe and Rust 2008; M.K.R., unpublished data).

By taking advantage of the excellent horizontal transfer of fipronil, a novel bait station was developed and tested against field populations of the Argentine ant. The novel bait station was referred to as a virtual bait station because the sucrose solution bait itself did not contain any toxicant. The virtual bait strategy exploits the ant's preference for sweet liquid bait and its intense recruiting behavior. Foragers were permitted to cross surfaces treated with diluted fipronil en route to the sucrose solution bait. The workers picked up a dose that exhibited delayed toxicity over 3–5 d. The fipronil was later transferred to nestmates by physical contact such as grooming and necrophoresis. In theory, the virtual bait is an ideal model for a baiting strategy against sweet-feeding ant species because it does not necessitate the toxicant being soluble enough to be formulated with water-based sugar bait.

The California least tern, *Sterna antillarum browni* Mearns, a migratory near-shore seabird, has been on federal and state endangered species lists since 1970 and 1971, respectively (Elliott et al. 2007). The California least tern nesting colony at White Beach on Camp Pendleton along the coast north of Oceanside, CA, covers ≈2.02 ha divided into square plots established by the wildlife biologist to study the birds, each plot being ≈900 m^2 (30 by 30 m). Because California least tern nests on the sandy ground of the beach, the chicks are particularly susceptible to attack by opportunistic ants such as *S. xyloni*, *Formica francoeuri* Bolton, and Argentine ants (Hooper et al. 1998; M.K.R., unpublished data). Argentine ants are the primary ant species on the White Beach California least tern nesting site. White Beach is an atypical habitat for Argentine ants because the soft sand of the beach does not provide adequate support for the ants to build nests and tunnels. Thus, nearly all of the ants nest in or adjacent to loose pieces of wood debris at the site, at the bases of plants and fence posts placed around the site to protect the birds from human intrusion. In addition, the soil near the sand beach is moist and compacted enough to allow ants to construct nests. The objective of this study was to develop and evaluate the virtual bait strategy as a potential method of reducing or suppressing Argentine ant populations at the endangered seabird nesting site.

Materials and Methods

Ants for Laboratory Testing. Argentine ants were collected from a citrus grove on the University of California, Riverside campus. Ant nests were excavated from the ground and transported to a laboratory chamber where they were extracted from the soil (Hooper-Bui and Rust 2000). Large laboratory colonies were maintained in plastic boxes (26.5 by 30 by 10 cm) with the inner sides coated with Teflon (fluoropolymer resin, type 30, DuPont Polymers, Wilmington, DE) to prevent ants from escaping. Each colony was provided with two or three artificial nests constructed from plaster-filled petri dishes (9 cm in diameter by 1.5 cm in height) formed with a cylindrical area (5 cm in diameter by 1 cm in depth) in the center of the dish to serve as a nesting space (Soeprono and Rust 2004). Colonies were provisioned with fresh water, 25% (wt:vol) sucrose solution, and freshly killed western drywood termites, *Incisitermes minor* (Hagen), and American cockroaches, *Periplaneta americana* (L.).

Laboratory Exposure Studies. The objective was to briefly expose ants to fipronil-treated surfaces so that ≈50% of the exposed ants died in 3–5 d. This delayed toxicity allowed ants to continue to recruit other foragers for as long as possible. To determine the amount of fipronil to apply to the inner surface of the polyvinyl chloride (PVC) pipe (15.5 cm in diameter by 30 cm in height), ants were forced to forage across a 30-cm-long strip of treated PVC pipe to obtain the sucrose solution. Before the experiment, an ant colony was deprived of sucrose solution and dead insects for at least 3 d. During this period, only water was provided. A narrow strip (2.5 by 30 cm; 75 cm^2) was cut from a section of PVC pipe. The inner surface of the strip was treated with an aqueous preparation of fipronil (Termidor 2 SC, BASF Corp., Research Triangle Park, NC) with a camel's-hair brush at a rate equivalent to 3.3, 5, 6.7, 8.3, or 10 μg cm^{-2} (active ingredient/surface area). Twenty drops of Tween 80 (Fisher, Fair Lawn, NJ) were added to the 100-ml fipronil preparations to prevent puddling and to ensure uniform coverage on the PVC strip. Termidor was mixed in water at a concentration such that 0.5-ml aliquots applied to the inner surface of a PVC pipe strip provided the required treatment rate. The strips were allowed to dry for 24 h. A small polystyrene weighing dish (4.5 cm in diameter) was attached at one end of the PVC strip with hot glue and filled with 25% sucrose solution. An ant was released at the other end of the strip, and it had to traverse the treated surface to obtain the sucrose solution. To ensure a straight passage by the ant across the treated surface, a 5 μl acetone solution of the synthetic trail pheromone *cis*-9-hexadecenal (10 μg/ml) was applied to the surface of the strip in a straight line using a disposable glass pipette (Greenberg and Klotz 2000). After the ant consumed the sucrose solution, it returned along the pheromone trail to its original release point. The average of the total exposure time going to and from the sucrose solution was 45.9 ± 1.9 s (mean ± SEM; n = 80). This procedure was repeated 10 times for each deposition rate. The ants (n = 10) were captured on their return and placed together in a petri dish (9 cm in diameter by 1.5 cm in height) provided with water. The number of dead ants was counted daily for 6 d. The efficacy of the treated PVC strips was reexamined using identical methods after aging them 1 wk under ambient laboratory conditions (22–25°C and 17–41% RH).

Because the size and manufacturer of the PVC pipe and the application method for fipronil were different from the 2007 study, the application rate of fipronil for the inner surface of the PVC pipe was determined again for 2008 and 2009 studies by using the similar method described previously. A strip (2.5 by 25 cm; 62.5 cm^2) was cut from a section of PVC pipe (10.5 cm in diameter by 25 cm in height). The inner surface of the strip was sprayed with an aqueous preparation of fipronil with an airbrush (Paasche Type UT, Paasche Airbrush Company, Chicago, IL) at a rate equivalent to 1.65, 3.3, 5, 6.6, and 8.3 $\mu g\ cm^{-2}$ (active ingredient/surface area). Ten ants were tested in each trial, and each trial was repeated five times per treatment. The ants for each trial were captured on their return from the sucrose solution and placed together in a petri dish (9 cm in diameter by 1.5 cm in height) and provided with water. The number of dead ants was counted daily for 5 d.

Effect of Dead Ants in Sucrose Solution. To determine whether the accumulation of dead ants in the sucrose solution dispenser of the virtual bait station affected the foraging behavior, sucrose solutions that had contained different numbers of dead ants were presented to laboratory ant colonies in choice studies. Aliquots (10 ml) of stock 25% (wt:vol) sucrose solution were transferred to seven glass scintillation vials (Kimble Chase Life Science and Research Products LLC, Vineland, NJ). Subsets of workers from a laboratory colony of Argentine ants were anesthetized using CO_2 and subsequently counted. Live Argentine ant workers (0 [control], 10, 50, 100, 200, and 300 ants) were placed in the sucrose solution vials, and the vials were capped. The vials were stored at room temperature (22–25°C) for 3 d. After all of the workers died, the vials were stored in a high humidity chamber (22–25°C and 75% RH) for another 4 d without the caps. The dead ants were removed from the prepared sucrose solution by filtering it through a disposable glass pipette plugged with glass wool. The filtered sucrose solution was stored in a refrigerator until tested.

Six small plastic feeders (detachable caps from disposable 1.5-ml microcentrifuge vials) were equally spaced along the edge (every 60°) of a plastic petri dish lid (9 cm in diameter). Approximately 100 μl of each sucrose solution/dead ant preparation was placed in each feeder, and the petri dish lid with all the feeders was then placed in the foraging area of an Argentine ant colony box. Foraging workers readily

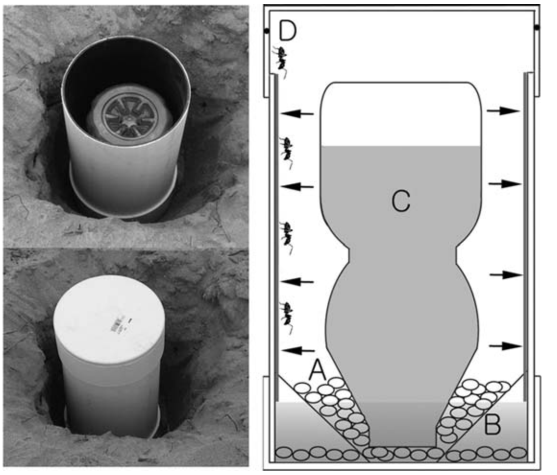

Fig. 1. Virtual bait station (2007 study). A layer of smooth rocks (A) and an inverted plastic cone (B) were placed in the bottom. A plastic bottle of 25% sucrose solution (C) with a hole in the cap was inverted in the rocks. The top of the pipe was capped and ants had access to the station through holes (D) in the top of the pipe. Thus, ants foraged across the fipronil treatments (arrows) to access the sucrose solution.

discovered the sucrose solutions, fed on them, and began recruiting nestmates. The petri dish lid was photographed every 5 min for 30 min until the trial was terminated. Based on the photographs taken, the number of ants feeding at each feeder was counted at 5-min intervals. Five different colonies of similar sizes (5–10 queens, brood, and 1,000–2,500 workers) were used, and the experiment was repeated four times for each colony. All laboratory colonies were equally provisioned with the six different sucrose solutions that previously had various numbers of dead ants in them (100 μl of each) ≈20–24 h before trials, allowing foragers to experience all different sucrose solution options before the actual experiments. Because foragers from a starved colony tend to start feeding on any sucrose solution regardless of their preference, the preexposure of foragers to all the different solutions were important to measure the accurate relative preference during the actual 30-min-long choice studies.

Field Study 2007. The virtual bait stations (Fig. 1) were modified from a bait station designed by Cooper and Daane (2007). However, in our design, the inner surface of PVC pipe (15.5 cm in diameter by 30 cm in height) was treated with fipronil, and the sucrose solution did not contain a toxicant. A layer of smooth rocks and an inverted plastic cone (10.5 cm in diameter, SoccerOne, Canoga Park, CA) were placed in the bottom. A 2-liter plastic bottle of 25% sucrose solution with a hole in the cap was inverted in the rocks. The inverted plastic cone allowed ants to walk between the inner surface of the PVC pipe to the sucrose solution. The top of the PVC pipe was capped and four equally spaced horizontal holes (8 mm in diameter) were drilled along the top of the pipe to permit access by ants to the sucrose solution. Thus, ants foraged across the fipronil treatments to access the sucrose solution.

Fourteen treated virtual bait stations (treatment rate, 6.7 or 8.3 μg cm^{-2}) were tested at White Beach, Camp Pendleton. The inner surface of bait station was painted with an aqueous preparation of fipronil by using an identical method described previously in the laboratory exposure studies. The sandy beach site was the nesting habitat for the endangered seabird California least tern from April to August. The Argentine ants attack nesting chicks on the beach and are believed to interfere with successful fledging of birds. A single bait station was placed in each of 14 plots (30 by 30 m). The stations were buried in the sand ≈28 cm in depth to avoid direct sunlight and keep them cool. Only the caps and entrance holes of the stations were visible. Twelve control plots (30 by 30 m) remained untreated.

To evaluate the efficacy of the virtual bait stations for ant control, estimates of the number of foraging ants in the baited and untreated plots were compared. The activity and number of ants were estimated by determining the amount of 50% (wt:vol) sucrose solution consumed by the ants over a 24-h period. Two monitoring tubes (Blue Max 50-ml polypropylene conical tubes, BD Biosciences, San Jose, CA) containing 45 ml of sucrose solution were placed in each plot along the centerline of rectangular plot, ≈20 m apart. The tubes were wedged diagonally in the sand to maximize the surface area of liquid available to the ants and reduce the risk of ants drowning. Each tube was covered with a yellow wooden cover (15 by 15 cm) to protect it from precipitation, wild animals, sunlight, and blowing sand. The amount of sucrose solution consumed by the ants was determined by measuring the weight loss from the tubes over 24 h, and then correcting for evaporation. The correction for evaporation was based on the weight loss from another set of monitoring tubes (evaporation check tubes; $n = 2$) with Teflon around the top of the tube that prevented ants from gaining access. Based on laboratory studies conducted by Reierson et al. (1998), Argentine ants consume on average 0.3 mg of sucrose solution per visit. This average consumption was used to estimate the number of ant visits to each tube over 24 h. The monitoring tubes were serviced at the same time of day (i.e., between 1000 and 1100 hours PST) throughout the study. The study site was monitored before treatment and 4, 8, 12, 18, and 22 wk after treatment.

At the end of 2007 field study, the bait stations were brought back to the laboratory and tested for their insecticidal activity. The PVC pipes were tested using similar methods described previously in the laboratory exposure studies. Ten ants were used for a trial and each trial was repeated three times. The control was prepared by allowing ants to walk on a clean PVC pipe strip of similar size. The number of dead ants was counted daily for 5 d.

Field Study 2008. To prevent the accumulation of dead ants in the sucrose solution dispenser at the bottom of the virtual bait stations, the original 2007 design was modified, making it possible to remove and replace the sucrose solution container and dispenser (Fig. 2). An inverted 473-ml cylindrical high density polyethylene bottle (Container and Packaging Supply Inc., Eagle, ID) was used as the sucrose solution container (Fig. 2E). Two bottle caps (28 mm in diameter) combined by melting, one with a hole (15 mm in diameter) on its top and the other with three holes (8 mm in diameter) on its side, served as the sucrose solution dispenser (Fig. 2F). An aluminum weighing dish (6 cm in diameter by 1.5 cm depth) was attached at the bottom of the dispenser to allow sucrose solution bait to be collected without spilling (Fig. 2I). The dispenser with the aluminum dish, an inverted plastic cone (10.5 cm in diameter), and a plastic petri dish bottom (9 cm in diameter by 1.5 cm in height) were connected with a plastic nut and bolt. The dispenser part (Fig. 2, dotted line inset) with the sucrose solution container was inserted into a section of PVC pipe (10.5 cm in diameter by 25 cm in height), and two holes (8 mm in diameter) were drilled along the top of the pipe, permitting ants to enter. The inverted plastic cone allowed ants to walk between the inner surface of the PVC pipe to the dispenser containing the sucrose solution. A plastic drain cap (11 cm in diameter, NDS, Lindsay, CA) and end cap (Grip Tip cap, 11.5 cm in diameter, Sioux Chief Mfg., Peculiar, MO) served as the bottom and top of the bait station, respectively.

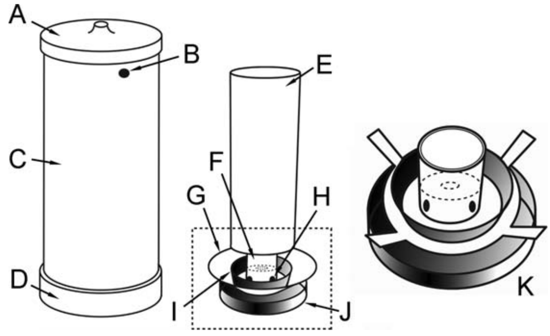

Fig. 2 Schematic diagram of the virtual bait station with exchangeable sucrose solution container and dispenser (2008 and 2009 study). For the 2008 study, the bait station housing consisted of a cap on top (A), PVC pipe (C) with access holes (B), and a cap on bottom (D). Exchangeable bait cartridges consisted of bait container bottle (E), fused bottle caps (F) with holes (H) as a dispenser, an inverted plastic cone (G), an aluminum weighing dish (I), and petri dish cover (J) at the bottom. For the 2009 study, the dispenser part (dotted line inset) was further modified so that the inverted cone (G) provided only four narrow bridges for foraging ants (K).

Twenty-six treated virtual bait stations (treatment rate, 1.65 μg cm^{-2}) were tested at White Beach (total of 13 plots [30 by 30 m] in the treated area with two bait stations per plot). The inner surface of bait station was airbrushed with an aqueous preparation of fipronil. Ant activities at both treated and untreated plots were monitored before treatment and 4, 8, and 10 wk after treatment. Two monitoring tubes containing 45 ml of sucrose solution were placed in each plot along the transect perpendicular to the trap line axis, ≈20 m apart. The bait cartridges (dispenser part and sucrose solution container) were removed from the bait station housing during 24-h monitoring, and replaced with clean ones (refilled with 200 ml of 25% sucrose solution) at each monitoring date.

Field Study 2009. In the 2008 field study, the dead ants tended to accumulate between the aluminum weighing dish and the inverted plastic cone where ants walked from the treated surface to the sucrose solution. The accumulation of the dead ants was probably detrimental to maintaining recruitment and foraging activity. Thus, in 2009, the dispenser part of bait cartridge was further modified to minimize the accumulation of dead ants. The shape of the existing inverted plastic cone was modified by cutting it so that it provided four narrow bridges (5–8 mm in width) for foraging ants (Fig. 2K). Because of the narrow bridges between the sucrose solution dispenser and the inner surface of PVC housing, most of the dying ants dropped to the bottom of the station instead of accumulating between the aluminum weighing dish and the inverted plastic cone.

Twenty-eight treated virtual bait stations (treatment rate, 1.65 μg cm^{-2}) were tested at White Beach (total of 14 plots [30 by 30 m] in the treated area with two bait stations per plot). The inner surface of bait station was airbrushed with an aqueous preparation of fipronil. Ant activities at both treated and untreated plots were monitored before treatment and 3, 6, and 9 wk after treatment, by using the methods described for 2008 field study. The bait cartridges containing sucrose solution were removed from the bait station housing during 24-h monitoring, and replaced with clean cartridges (refilled with 200 ml of 25% sucrose solution) at each monitoring date.

Data Analysis. For the laboratory sucrose solution feeding studies, the number of ants feeding at each sucrose solution was pooled over each 30-min period, and the pooled data were used for further analyses. Heterogeneity chi-square analyses were performed to decide whether the data from the series of six different observations (i.e., 5, 10, 15, 20, 25, and 30 min) were sufficiently uniform to be pooled (Zar 1999). Because Argentine ant foragers spent 145.6 ± 9.9 s (mean ± SEM; $n = 80$) feeding on the sucrose solution per visit (D.-H.C., unpublished data), we assumed that a significant portion of ants feeding at two consecutive observations (300 s apart) were different individuals. Statistical analysis of multiple-choice feeding preference experiments is problematic because consumption within a trial violates analysis of variance (ANOVA)'s independence assumptions (Roa 1992, Manly 1993, Lockwood 1998). Following the recommendation of Lockwood (1998), the relative preferences (i.e., ratios of the number of foragers in a particular sucrose solution to the total number of foragers) were analyzed using a multivariate Hotelling's T^2 test (PROC IML, SAS Institute 2006). If the null hypothesis (i.e., all the sucrose solutions were preferred equally) was rejected, we performed pairwise comparisons to determine which of the components deviated most significantly (Lockwood 1998).

For the virtual bait station field studies, the pretreatment counts of the control and treatment plots were compared with a Wilcoxon rank-sum test (Analytical Software 2005). Because the pretreatment counts for control and treatment plots were quite different (especially in 2007 and 2008 studies), the count data at both plots were converted to percentages of corresponding pretreatment average values. Then, the efficacy of virtual bait stations was determined by comparing data pooled from all posttreatment monitoring dates with a Wilcoxon rank-sum test (Analytical Software 2005). This analysis also was conducted for 2009 data for consistency, despite the similar pretreatment counts between control and treatment. For 2008 and 2009, we recorded the presence or absence of live foraging ants at monitoring tubes when they were retrieved, then the proportions of monitoring tubes without foragers were compared between control and treatment plots using a Fisher exact test (Analytical Software 2005).

Results

Laboratory Exposure Studies. For 2007 study, fipronil deposits of 5, 6.7, 8.3, and 10 μg cm^{-2} on the PVC strip provided 100% kill within 5 d. After aging 1 wk, the efficacy of the 5 μg cm^{-2} deposit provided only 40% kill within 4 d. Exposure to 10 μg cm^{-2} provided faster kill, with 100% mortality after 2 d. Exposure to 6.7 and 8.3 μg cm^{-2} resulted in delayed kill, with ≈50% mortality between 3 and 5 d. Based on these results, the inner surface of the virtual bait station was treated with fipronil at a rate equivalent to 6.7 or 8.3 μg cm^{-2} (total of 14 stations, eight treated with 6.7 μg cm^{-2} and six treated with 8.3 μg cm^{-2}). For the PVC pipe used in 2008 and 2009, fipronil deposits of 1.65 and 3.3 μg cm^{-2} on PVC strips resulted in delayed mortality between 3 and 5 d. Thus, the inner surface of the virtual bait station was treated with fipronil at a rate equivalent to 1.65 μg cm^{-2}.

Effect of Dead Ants in Sucrose Solution. The presence of dead ants in the sucrose solution negatively affected the preference of foraging ants. Overall, significantly more foragers were found feeding on the control than all other treatments ($T^2 = 111.4$; df = 5, 15; $P < 0.0001$) (Fig. 3). Sucrose solutions with more dead ants were less preferred than sucrose solutions with fewer dead ants, indicating feeding and subsequent recruitment of foragers were clearly deterred or inhibited by dead ants in the sucrose solution (Fig. 3).

Field Study 2007. The initial number of ants in the control section was significantly higher than in the treatment section (Wilcoxon rank-sum test: $z = 3.4$, $P < 0.001$) (Table 1). At week 2, thousands of dead ants were found in the sucrose solution at the bottom of virtual bait stations, and no active foragers were found in the bait station upon monitoring. The overall posttreatment foraging activity levels obtained from all five sampling dates were not significantly different between the control and the treated sections (Wil-

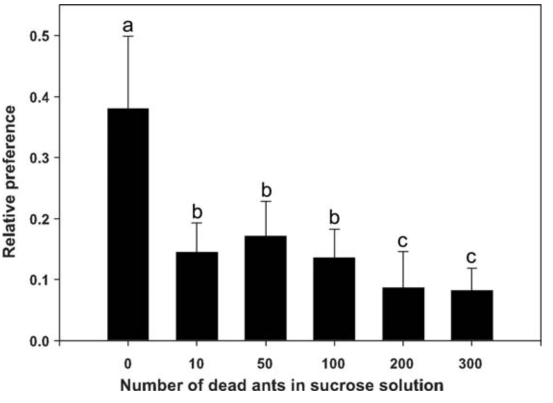

Fig. 3. Relative preference of Argentine ant foragers for sucrose solutions that contained various numbers of dead ants. Values represent means ± SD. Bars labeled with the different letters are significantly different (Hotelling's T^2 test; $\alpha = 0.05$).

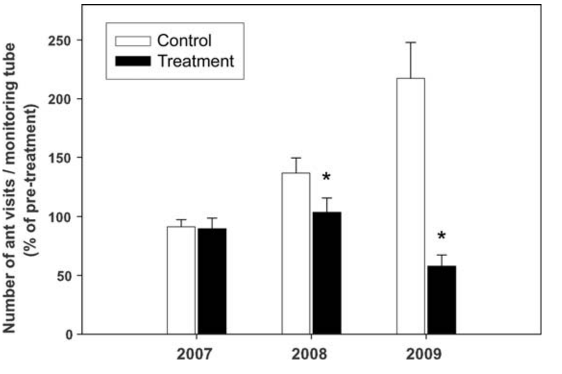

Fig. 4. Efficacy of virtual bait stations against Argentine ants at White Beach. The average numbers of ant visits per monitoring tube are expressed as percentage of pretreatment value, and data are pooled from all posttreatment sampling dates. Values represent means ± SEM. The bars marked with asterisks are significantly different from corresponding controls (Wilcoxon rank-sum test; $\alpha = 0.05$).

coxon rank-sum test: $z = 1.8$, $P = 0.08$) (Fig. 4). At the end of 2007 field study, the inner surface of PVC bait housing still maintained its insecticidal activity, providing 40–90% kill of foragers within 5 d in the laboratory, whereas the control showed 0–10% mortality.

Field Study 2008. The exchangeable cartridges partially improved the performance of the bait stations, allowing ants to actively forage in some bait stations even 10 wk after the initial setup. Most of the dead ants accumulated between the inverted plastic cone and the aluminum weighing dish containing sucrose solution. However, some dead ants were still found in the sucrose solution, and contamination of the sucrose solution with decomposing ants and associated fungus occurred.

The initial number of ants in the control section was significantly higher than in the treatment section (Wilcoxon rank-sum test: $z = 3.5$, $P < 0.001$) (Table 1). The overall posttreatment foraging activity level obtained from all three sampling dates was significantly lower in the treatment section than in the control (Wilcoxon rank-sum test: $z = 2.6$, $P = 0.009$) (Fig. 4). The proportion of monitoring tubes without ants remained higher in the treated section (21–50%) than in the control (0%) throughout the study except for week 8, in which 11 and 0% of monitoring tubes did not have foraging ants in the treated section and control, respectively (Fig. 5A).

Field Study 2009. The modified bait dispenser with four narrow bridges improved the efficacy of the bait stations significantly by preventing many of the dead ants from accumulating in the bait dispenser. Most of the dead ants were found at the bottom of bait station, leaving the sucrose solution with fewer dead ants.

The initial numbers of ant visits to the monitoring vials in both sections were similar (Wilcoxon rank-sum test: $z = 0.1$, $P = 0.92$) (Table 1). The overall posttreatment foraging activity level obtained from all three sampling dates was significantly lower in the treatment section than in the control (Wilcoxon rank-sum test: $z = 5.5$, $P < 0.001$) (Fig. 4). The proportions of monitoring tubes without foragers were similar between treated (29%) and untreated (26%) sections before the treatment (Fig. 5B). After the treatment, however, the proportions of monitoring tubes without ants decreased and remained low (0–17%) in the untreated control, whereas those in the treated sections increased and remained higher (43–64%) (Fig. 5B).

Table 1. Foraging activity of Argentine ants at White Beach based on the number of ant visits/monitoring tube

Yr	Section	Pretreatment	Posttreatment at wk[a]				
			4	8	12	18	22
2007	Control	100 (56,073)	81.7 ± 11.5 (45,791)	73.4 ± 8.1 (41,173)	78.3 ± 7.1 (43,883)	121.9 ± 17.2 (68,345)	101.9 ± 17.5 (57,150)
	Treatment	100 (23,885)	83.2 ± 19.8 (19,880)	73.6 ± 16.1 (17,581)	105.2 ± 17.9 (25,133)	150.1 ± 24.1 (35,856)	36.5 ± 10.8 (8,719)
			4		8	10	
2008	Control	100 (41,455)	139.8 ± 25.0 (57,969)		138.1 ± 19.5 (57,247)	131.8 ± 23.2 (54,652)	
	Treatment	100 (14,078)	79.8 ± 15.9 (11,241)		147.6 ± 30.0 (20,776)	83.5 ± 11.0 (11,756)	
			3		6	9	
2009	Control	100 (15,574)	103.1 ± 31.7 (16,059)		310.6 ± 62.5 (48,373)	237.9 ± 53.2 (37,046)	
	Treatment	100 (16,691)	32.0 ± 10.8 (5,339)		58.9 ± 12.9 (9,829)	82.8 ± 22.7 (13,827)	

[a] Value (mean ± SEM) is expressed as a percentage of corresponding pretreatment count. Averages of actual count data are presented in parentheses.

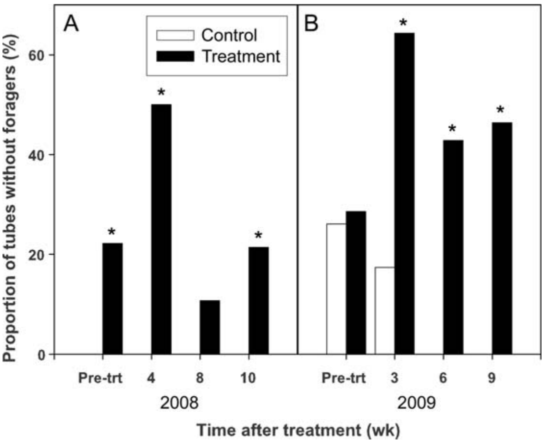

Fig. 5. The proportions of monitoring tubes without ants foraging sucrose solution. Bars labeled with asterisks are significantly different with corresponding controls (Fisher exact test; $\alpha = 0.05$).

Discussion

The control of Argentine ants remains a serious problem with no single specific strategy being consistently effective (Rust et al. 2003, Silverman and Brightwell 2008). Barrier applications and spot sprays with fipronil have been reported to provide excellent control around structures for up to 12 wk (Klotz et al. 2008b). However, the use of fipronil away from structures is not permitted. Sprays in environmentally sensitive areas are typically discouraged and baits are frequently recommended as an alternative approach to control ants in these areas (Klotz et al. 1997). Unfortunately, development and practical use of commercial baits for Argentine ant control have not been very successful for a variety of reasons including insufficient amount of bait applied in the field, decrease of bait palatability over time, excessive decrease or increase of toxicant concentration in the baits, and low solubility of some slow-acting toxicants in aqueous bait formulations (Rust et al. 2003, Silverman and Brightwell 2008).

Similar to many other dolichoderine ant species foraging on extrafloral nectaries and tending hemipterans that produce honeydew, Argentine ants will forage on sugary solution the entire year (Rust et al. 2000, Klotz et al. 2008a). Consequently, the ants will recruit to bait stations filled with sucrose solution even when the seasons and reproductive cycles of the ant colony change. The virtual bait station exploits the strong feeding preference of the Argentine ants for sugars and carbohydrates. The virtual bait station also avoids the problems associated with formulating insecticides into aqueous sucrose solutions, especially those with low water solubility.

In 2007, the virtual bait stations were not successful in significantly reducing the foraging activity in the treated plots. In particular, foragers stopped visiting most of the virtual bait stations within ≈2 wk after the initial setup. Dead ants accumulated in the sucrose solution collected at the bottom of the virtual bait station and were suspected to be the cause of the observed feeding deterrence. The sucrose solution preparations that contained dead ants ($n > 50$) for 1 wk were more acidic than the control sucrose solution (D.-H.C., unpublished data). However, neutralization of the sucrose solution with NaOH only partially restored its palatability to ant foragers (D.-H.C., unpublished data). Thus, acidification of sucrose solution associated with dead ants or fermentation is at least in part responsible for the decreased foraging activity. However, because worker ants have various alarm and/or defensive compounds (Vander Meer and Alonso 1998), it is possible some of these chemicals emanating from dead ants may also be responsible for the decreased feeding activity.

At the end of 2007 field study, the bait stations were brought back to the laboratory and tested for insecticidal activity. The inner surface of PVC pipe still provided 40–90% kill of foragers within 5 d. Even though it was not determined whether the fipronil deposit on the inner surface was as effective as it had been originally, the insecticidal effect of the fipronil on the inner surface of PVC was reasonably well maintained over 22 wk. In addition to the relatively cool temperatures at the study site, the PVC container buried in the sand protected the treated surfaces from exposure to sunlight, precipitation, and heat, preventing the rapid breakdown of the fipronil deposits. Sustained insecticidal effect of fipronil in the bait station is particularly important because fipronil applied on exposed soil surface is highly sensitive to photolysis, especially at temperatures of >32°C (Connelly 2001, Gunasekara et al. 2007).

The field study in 2008 showed that the exchangeable bait cartridge used in the new virtual bait station permitted ants to visit the bait station for an extended period of time (≈10 wk). This improvement is particularly important because it would enhance the impact of the virtual stations by exposing more foragers to the fipronil deposit. A significant reduction in ant activity at the treated section was detected in 2008 at White Beach. However, the pretreatment foraging activity was much higher in controls than in the treatment plots, and this may have affected the results. The persistent low foraging activity observed in the treated plots in the 2008 study may be, in part, due to invasions of ants from adjacent populations. Even with the elimination of a colony adjacent to the bait stations, immigration from nearby populations may occur (Vega and Rust 2003). In fact, Argentine ants are known to be able to forage considerable distances (i.e., >60 m) (Vega and Rust 2003, Silverman and Brightwell 2008), and active foraging trails of Argentine ant were observed outside of the baited area in the 2008 study.

In 2009, virtual bait stations were further improved by narrow bridges between the sucrose solution dispenser and the treated inner surface of bait housing. The bait stations provided >50% reductions in the numbers of ants foraging in the treated area for at least 9 wk. It is possible that the narrow bridges provided additional challenges to intoxicated ants in the bait station, making them drop to the bottom without accumulating around the sucrose solution dispenser. The narrow bridges remained free of dead ants for a longer period, while providing access to other live foragers to the sucrose solution bait. This design also helped to minimize the contamination of sucrose solution with dead ants.

Further improvements need to be made to develop a commercial prototype of the virtual bait station. First, installation of bait stations around residential structures would be easier and more practical with smaller stations. Small bait stations could be hidden in garden vegetation or hedges. Furthermore, this will also help protect bait stations from direct sunlight, heat, or pets without burying it in the ground. Second, a replaceable or refreshable insecticide-treated inner surface of the bait station might improve the longevity of the bait station. For example, a thin plastic film treated with fipronil could be rolled and inserted in the bait station housing. The insecticide-treated film could then be easily removed and replaced with a new one as the old film loses its insecticidal activity. This would eliminate the need for replacing entire bait stations, thereby improving the economics of the virtual baiting strategy by reusing the bait station housing already buried in the ground or set close to existing foraging trails.

This study demonstrated the potential of the virtual baiting system to suppress or decrease foraging activity of Argentine ant populations in environmentally sensitive sites. The virtual bait station allows the use of active ingredients with low solubility in water and minimizes the possibility that insecticides might contaminate the environment. Additional improvements are still needed on the station design.

Acknowledgments

We are grateful to Donald A. Reierson (University of California, Riverside) for assistance in bait station manufacture. We thank L. Greenberg for reading an earlier version of the manuscript. We are indebted to Sherri Sullivan and Tiffany Shepherd for the assistance at Camp Pendleton. This work was partially supported by a Carl Strom/Western Exterminator Scholarship, a Pi Chi Omega Scholarship, a Bayer Young Scientist of the Year 2008 Scholarship (to D.-H.C.), and the Naval Facilities Engineering Command.

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Received 27 April 2010; accepted 22 June 2010.