Efficacy of Simulated Barrier Treatments Against Laboratory Colonies of Pharaoh Ant

GRZEGORZ BUCZKOWSKI,[1, 2] MICHAEL E. SCHARF,[3] CATINA R. RATLIFF,[1]
AND GARY W. BENNETT[1]

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ABSTRACT Five selected insecticides were applied to four substrates and evaluated in laboratory studies for repellency and toxicity against the Pharaoh ant, *Monomorium pharaonis* (L.). We tested both repellent and nonrepellent formulations on outdoor (concrete and mulch) and indoor (ceramic and vinyl) substrates. Repellency was evaluated using a behavioral bioassay in which colonies were given a choice to leave the treated zone and move into empty nests provided in the untreated zone. We used a novel experimental design whereby ants walked on a Slinky coil suspended from a metal support frame, thus permitting a long foraging distance with a minimum use of space and resources. Cypermethrin, a repellent pyrethroid insecticide, resulted in colony budding, although the response was delayed. Toxicity of insecticides was evaluated as worker, queen, and brood mortality. The most effective treatment was fipronil, which provided 100% reduction in pretreatment activity by 2 d posttreatment on both concrete and mulch. Chlorfenapyr was highly effective on both outdoor and indoor substrates. Significant substrate effects were observed with insecticides applied to nonabsorbent substrates (ceramic tile), which performed better than insecticides applied to absorbent substrates (vinyl tile). Other highly absorbent materials (mulch and concrete), however, did not reduce insecticide efficacy. This is because ants relocated nests into and/or under these attractive nesting materials, thus increasing their exposure to toxic insecticide residues. Our results demonstrate efficacy of nonrepellent liquid insecticides as indoor treatments for the control of Pharaoh ants and possibly as exterior perimeter treatments.

KEY WORDS *Monomorium pharaonis*, Pharaoh ant, repellency, residual insecticide

THE PHARAOH ANT, *Monomorium pharaonis* (L.), is an introduced species that exhibits several tramp ant characteristics such as generalist diet, polygyny, polydomy, large colony size, reproduction by budding, and close association with humans (Passera 1994). These traits make colonies of Pharaoh ants successful invaders of human built structures (Edwards and Baker 1981, Edwards 1986) and also make them extremely difficult to eradicate. Of the habits listed above, reproduction by budding (sociotomy) is perhaps the most critical to the success of Pharaoh ants. Although the majority of ant species disperse and start new colonies by individual females after a mating flight, Pharaoh ants mate in the nest and colony reproduction occurs by fragmentation of mature colonies (Edwards 1986). During budding, a reproductively competent colony fragment migrates to a new location to establish a new nest (Peacock et al. 1955, Vail and Williams 1994), which may or may not remain in association with the parent colony. Thus, new colonies are created independently of gyne production and may be initiated at any time. Traditionally, part of the success and persistence of the Pharaoh ant had been attributed to its frequent budding habits (Edwards 1986). Colony fragmentation may occur due to a wide range of biotic and abiotic factors; however, only limited information exists on the factors responsible for inducing the budding behavior. Possible factors include overcrowding, response to weather (seasonal changes in a structure's central heating and cooling system), physical disturbance, dietary change (depletion of or discovery of new resource), or chemical disturbance (application of a repellent pesticide). Traditionally, applications of repellent spray insecticides have been alleged to be the major factor promoting fragmentation of colonies (Green et al. 1954, Lee et al. 1999). However, no detailed experimental data exist to either support or refute this claim. In addition, residual insecticides are usually ineffective for complete colony elimination because they only affect foraging workers and fail to reach reproductive individuals (Lee et al. 1999).

Recently, new classes of nonrepellent pesticide chemistries have become available for urban pest control. The phenyl pyrazoles (fipronil) and the structurally similar pyrroles (chlorfenapyr) are both nonrepellent residual insecticides that have proven

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[1] Department of Entomology, Purdue University, West Lafayette, IN 47907-2089.

[2] Corresponding author, e-mail: gbuczkow@purdue.edu.

[3] Entomology and Nematology Department, P.O. Box 110620, University of Florida, Gainesville, FL 32611-0620.

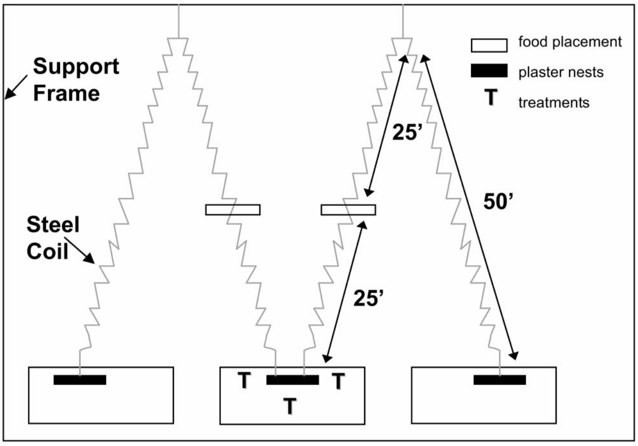

Fig. 1. Experimental design. Three plastic trays were connected by steel coils suspended from a metal support frame. The length of the coil between any two trays was 100 feet. Nest containing the colony was placed in the center tray and surrounded by treated surfaces. Alternative nesting trays were provided on either side.

effective against a variety of pests of public and medical importance (Scott and Wen 1997, Ameen et al., 2000).

The goals of this study were two-fold. Our main objective was to compare the efficacy of barrier treatments of reportedly nonrepellent insecticides (fipronil and chlorfenapyr) to ones that are thought to be repellent (synthetic pyrethroids). We hypothesized that nonrepellent insecticides will be associated with greater mortality via direct and indirect (horizontal transfer) exposure to the active ingredient (e.g., Buczkowski and Schal 2001). In contrast, repellent treatments will cause lower mortality due to colony relocation (budding) once the insecticide is detected. Our second objective was to evaluate the efficacy of residual insecticides on a variety of outdoor and indoor substrates. Previous studies emphasized the importance of surface type on insecticide efficacy (Chadwick 1985, Knight and Rust 1990, Osbrink and Lax 2002) with nonporous substrates generally providing better, longer lasting control. We examined four substrates likely encountered by foraging Pharaoh ants: two outdoor substrates (concrete and mulch) and two indoor substrates (nonabsorptive ceramic and absorptive vinyl tile).

Materials and Methods

Laboratory Pharaoh ant colonies were maintained at constant temperature and humidity in an environmentally controlled rearing and testing room (25 ± 1°C, 65% ± 10% RH). Colonies were reared in 38 by 50-cm Fluon-coated trays (DuPont Polymers, Wilmingtom, DE) on a regular diet of 10% sucrose, whole cricket, peanut oil, and boiled egg yolk. Nests were plastic dishes filled with moist plaster. Experimental colony units were obtained by placing empty nests (9 cm in diameter) in colony rearing trays for voluntary colonization by the ants. The nests were covered with red acetate sheets to create the darkened conditions preferred by the ants. Plastic spacers were inserted between the nest floor and the covering to restrict the ants to a monolayer to facilitate counting of the various castes and developmental stages. When the nests were observed to contain ≈20 queens, 80–90 1-cm^2 grid squares of brood (counted by overlaying a grid over the nest and marking the outline of the brood), and ≈4,000 workers, single plates were transferred to the center tray of the experimental configuration shown in Fig. 1.

The experimental setup consisted of three 38 by 50-cm Fluon-coated plastic trays connected by steel (Slinky, James Industries, Inc., Hollidaysburg, PA) coils suspended from a metal support frame (Fig. 1). The length of the coil between any two trays was 100 feet, and previous work in our laboratory has shown that Pharaoh ants will routinely traverse this distance without difficulty (Ratliff and Bennett 2003). Empty nests were placed in trays adjacent to the center tray. To promote initial exploration of the center tray and the coils, food was provided mid-way (25 feet) along the coils immediately adjacent to the center plate. Food placements consisted of boiled egg yolk, peanut oil, 10% sucrose, and whole cricket provided three times a week.

After a 7-d acclimation period, treated substrates were placed on three sides of the nest in the center tray, 3 cm from the nest. We tested two types of outdoor substrates and two types of indoor substrates. Outdoor substrates were concrete pavers (20 by 10 by 4 cm in height) or hardwood mulch (Western White Wood Mulch, Menards Inc., Eau Claire, WI; arranged in aluminum foil trays 20 by 10 by 4 cm in height). Indoor substrates were ceramic (i.e., nonabsorptive surface) and vinyl (i.e., absorptive surface) tile, both 10 by 10 cm by 0.5 cm in height. Five different liquid spray insecticides were examined (Table 1), although not all insecticides were tested on all four substrates.

Table 1. Insecticides and active ingredients used in the study

Insecticide trade name	Active ingredient	Insecticide class	Manufacturer	Application rate ($\mu g/cm^2$)	Substrate tested on
Cinnamite	Cinnamaldehyde	Aldehyde	Mycotech, Butte, MT	12,973.0	Ceramic, vinyl
Demon EC	Cypermethrin	Type II pyrethroid	Syngenta Crop Protection Inc., Greensboro, NC	3.0	Ceramic, vinyl
Phantom	Chlorfenapyr	Pyrrole	BASF Corp, Research Triangle Park, NC	34.5	Concrete, mulch, ceramic, vinyl
Talstar F	Bifenthrin	Type I pyrethroid	FMC Corp., Philadelphia, PA	2.6	Concrete, mulch
Termidor SC	Fipronil	Phenylpyrazole	Aventis Environmental Science, Montvale, NJ	3.7	Concrete, mulch

The insecticides were applied to the top surface of all substrates by using a plant mister and allowed to dry for 4 h. The treatments remained in place for the entire length of the study. All insecticides were applied at label rates specified by the manufacturers and in equal volumes. Water-treated substrates were used as controls. Three replicates of each treatment were performed, and assays were run for 6 wk (42 d). Observations were made on day 2, 4, and 7 and subsequently once a week until day 42. Collected data consisted of 1) the presence or absence of colony relocation behaviors (defined as movement of reproductively competent colonies into side trays), 2) worker foraging activity, 3) worker mortality, 4) queen mortality, and 5) brood mortality. We define reproductively competent colonies as a group of 50 or more workers accompanied by at least one queen and/or brood (Peacock et al. 1955). In the Pharaoh ant, queens are not essential for successful foundation of new colonies because workers can rear new reproductives from existing brood (Vail and Williams 1994). Worker foraging activity was monitored by counting foragers crossing a marked line on all four 50-foot sections of the Slinky coil. The marks were 20 feet from the pans. The number of workers that crossed the mark in 5 min was recorded, and all counts were done before the ants were given food. Worker and queen mortality was assessed by aspirating dead individuals from the pans and counting them at the end of the 6-wk study. For workers, results are normalized to an assumed starting number of 4,000 workers. Brood mortality was estimated by counting the number of 1-cm^2 squares of brood at the beginning and end of the study. For queens and brood results are normalized to starting quantities.

Statistical analyses were performed in SAS 8.1 (SAS Institute 2002) and consisted of analysis of variance (ANOVA) (PROC ANOVA) on mean ant numbers by substrate category. A separate ANOVA test was performed for each of the four parameters measured (foraging activity, queen mortality, worker mortality, and brood mortality) and for each substrate category (outdoor versus indoor). Thus, eight separate ANOVA tests were performed: four for outdoor substrates and four for indoor substrates. Each ANOVA was followed by the least significant difference (LSD) *t*-test to test for significant differences between means. The level of significance was set at $\alpha = 0.05$.

Results and Discussion

Colony Movement. Workers frequently traversed the distance between nesting and alternative trays, and small groups of workers (50–100 individuals) occasionally explored and/or colonized side nests. In fact, all replicates in all treatments had small groups of workers nesting in alternative trays at some point during the study. However, no queens and/or brood were observed in side trays, with the exception of treatments involving Demon (cypermethrin; a repellent type II pyrethroid). Reproductively competent colony fragments colonized alternative trays in three of three Demon replicates involving vinyl tile, and one of three Demon replicates involving ceramic tile. However, colonies did not migrate to side trays until day 35. It remains unknown why colonies showed this delayed budding behavior, long after the initial exposure to insecticide. Other researchers, too, reported delayed budding with pyrethroid insecticides (Knight and Rust 1990). Perhaps budding occurs only after a certain threshold level of colony mortality is reached. Worker numbers steadily declined from day 1 to day 35, and excessive mortality by day 35 may have signaled a need to abandon the original nesting site in favor of one that offered greater protection from toxic insecticides. Nest and/or colony relocation seems to be a common behavior in ant colonies exposed to toxic chemicals. Imported fire ant *Solenopsis invicta* (Buren) colonies relocated their nests after insecticide applications (Collins and Callcott 1995) and Argentine ants, *Linepithema humile* (Mayr) avoided nesting in areas containing toxic aromatic cedar mulch (Meissner and Silverman 2003). Nest relocation may help colonies survive insecticide treatments and may be partly responsible for the difficulty in eradicating established colonies. Other pyrethroid insecticides, including cypermethrin and permethrin, also have been shown to be repellent to ants (Knight and Rust 1990). In temperate climates, Pharaoh ants nest mostly indoors; however, they also may forage outside in warmer climates (Oi et al. 1994). Thus, repellent pyrethroid insecticides should not be used for eradication of indoor colonies of Pharaoh ants. Such insecticides may, however, be useful in creating a repellent and potentially effective barrier to exclude Pharaoh ants from entering buildings in warmer climates.

Despite the relatively low incidence of budding into side trays, entire colonies were observed to readily

Table 2. Summary of observations indicating migration from original nests into or under treated substrates

Insecticide	Brick	Mulch	Ceramic	Vinyl
Control	(3/3)	(3/3)	(3/3)	(3/3)
Termidor SC (fipronil)	(0/0)*	(0/0)*	N/A	N/A
Talstar F (bifenthrin)	(1/3)	(0/3)	N/A	N/A
Phantom (chlorfenapyr)	(2/3)	(3/3)	(2/3)	(1/3)
Cinnamite (cinnamaldehyde)	N/A	N/A	(3/3)	(3/3)
Demon EC (cypermethrin)	N/A	N/A	(1/3)	(2/3)

Number of replicates where relocation occurred is provided in parentheses ($n = 3$). N/A, not applicable.

* Rapid onset of mortality prevented assessment of colony movement.

relocate into mulch and under pavers, ceramic, and vinyl tile (Table 2). Other tramp ant species also have been shown to relocate colonies into alternative substrates. Meissner and Silverman (2001) demonstrated that Argentine ants and odorous house ants, *Tapinoma sessile* (Say), abandoned plaster nests and colonized cypress mulch in choice assays. It remains unknown whether nesting in the vicinity of the treated substrates promoted or impeded mortality. On the one hand, colonies that relocated were in proximity to toxic insecticides. However, the undersides of pavers and tiles, as well as the bottom layer of mulch were untreated, which may have allowed some protection. Alternatively, ants could have become contaminated with insecticide residues in the process of exploring alternative nesting locations and moving brood over the treated substrates. Furthermore, after colonizing the underside of pavers, mulch, and tile, workers were still readily observed exploring the top surface of all substrates.

The differential relocation behaviors outlined in Table 2 can serve to provide an indirect measure of relative repellency. Both control and Cinnamite treatments were associated with colony relocation in all replicates, demonstrating lack of repellency by these treatments. Termidor, a nonrepellent material, acted so rapidly that no assessments on colony relocation could be made. However, our observations indicated that workers readily explored Termidor-treated mulch and pavers, thus confirming fipronil to be a nonrepellent insecticide. Chlorfenapyr also resulted in ants relocating from their original nests and into the treated substrates, also demonstrating a lack of repellency by this insecticide. Both Talstar, a type I (non-α-cyano) pyrethroid and Demon, a type II (α-cyano) pyrethroid, prevented colony relocation in most assays. The absence of colony relocation with Talstar and Demon suggests moderate repellency by these materials. It is possible, however, that other pyrethroid insecticides will be less repellent to Pharaoh ants.

In the current study, we used the nest relocation behavior as an indirect method of estimating insecticide repellency. This method provides information at the colony level, i.e., it suggests that a collective decision is made by numerous individuals of different castes. Other studies have used more direct methods that provide information on the individual level, such as counting the number of foragers crossing an imaginary line along a foraging trail (Knight and Rust 1990). Ants that reached the line had crossed the barrier and were thus not repelled. Although this methodology offers a direct assessment of repellency, its potential drawback is the underestimation of repellency due to rapid knockdown and/or mortality caused by some insecticides. Knight and Rust (1990) avoided this potential problem by normalizing counts to the number of ants surviving the treatments at each count. This method requires that all surviving ants be counted at each sampling period. Therefore, it is labor-intensive and may not be always practical, such as when colonies nest in mulch. An advantage of our methodology is that there is no need for exact worker counts at each sampling period. A potential disadvantage is the assumption that lack of movement indicates repellency. Lack of movement may only indicate preference to occupy the original nest versus the alternative substrate provided.

Foraging Activity. The toxicity of various insecticides was determined by observing changes in colony foraging behavior, i.e., the number of workers crossing a line on the Slinky coil. Foraging activity decreased significantly for all treatments relative to controls, except for Cinnamite, where worker counts were often higher than controls (Fig. 2). Of all insecticides tested, Termidor was the most effective at reducing ant foraging activity. Pretreatment activity declined by 100% by 2 d posttreatment on both concrete and mulch. Phantom was also highly effective on both outdoor and indoor substrates. On outdoor substrates, Phantom showed >80% reduction of pretreatment foraging activity by 2 d posttreatment. On indoor substrates, it provided reduction in the range of 70–100%. Phantom showed differences between indoor substrates, performing slightly better on ceramic tile, where almost 100% control was maintained throughout the 6-wk period. Foraging activity also decreased in the controls (Fig. 2). We have consistently observed this effect with Pharaoh ants in various experimental setups, and other researchers also reported declines in foraging activity in controls (Knight and Rust 1990). The decline in foraging activity may have several underlying causes. First, initial foraging activity may have been high due to the ants exploring the newly discovered foraging space and marking the territory with species-specific home range pheromones. Such behavior is common in other ant species (Aron et al. 1990, Mayade et al. 1993). Second, ants may have become satiated with the food source. To minimize this effect, we performed forager counts at least 24 h after colonies were provided with food.

In treatments involving outdoor substrates all treatments had significant, but similar impacts on average cumulative foraging activity over the entire 6-wk period (Fig. 3A). Also, no significant differences occurred between concrete and mulch substrates in cumulative foraging activity. In treatments involving indoor substrates (Fig. 3B), Phantom and Demon had significant impacts on cumulative foraging activity, with Phantom performing significantly better than

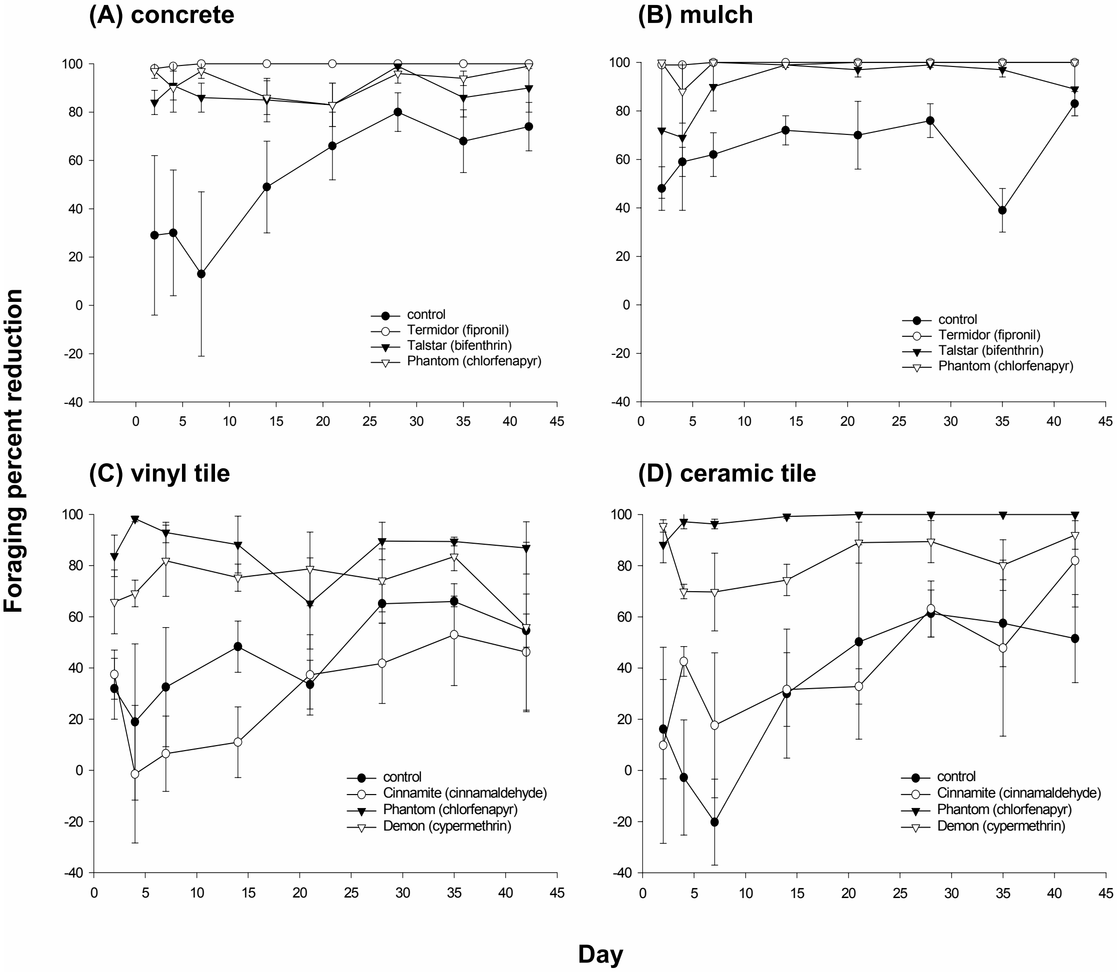

Fig. 2. Average (±SEM) percentage of reduction in worker foraging activity in response to various treatments at 2, 4, 7, 14, 21, 28, 35, and 42 d posttreatment ($n = 3$). Shown in A and B are results for outdoor substrates, and in C and D, indoor substrates. Data shown were normalized to pretreatment counts.

Demon on both vinyl and ceramic tile. The results for Cinnamite were not different from the controls. In fact, cumulative foraging activity for Cinnamite was higher than the controls. Based on comparisons of percentage of reductions in foraging activity over time (Fig. 2) versus cumulative foraging activity (Figs. 3A and 4A), it seems that monitoring of foraging activity reductions would suffice for comparisons of cumulative foraging activity by insecticide treatments.

Worker Mortality. Based on previous research (Ratliff 2003), starting worker numbers were normalized to 4,000. By 6 wk, worker numbers increased in controls, with mulch showing the greatest increase. In treatments involving outdoor substrates, Termidor had the most impact on worker numbers. It caused 99% mortality on concrete and 100% mortality in mulch by 6 wk. Talstar mortality ranged from 40 to 50%, whereas Phantom mortality was different between substrates. Phantom effects on pavers were similar to Talstar, whereas Phantom in mulch performed similarly to Termidor. On indoor substrates, Phantom was the most effective insecticide. It reduced the number of workers by 83% on ceramic tile and 20% on vinyl tile. Demon was significantly less effective than Phantom on ceramic tile and caused only 33% reduction in worker numbers. Demon was ineffective at reducing colony size on vinyl tile and colonies grew by 27%. Similarly, Cinnamite was ineffective on both substrates.

Queen Mortality. Impacts of treatments on queen mortality are shown in Figs. 3C and 4C. Significant substrate effects were observed in queen reduction in controls. This was likely caused by the difficulty in assessing weekly queen numbers in paver and mulch assays, due to the fact that colonies moved under pavers and into mulch in control and nonrepellent insecticide assays. Termidor was highly effective at killing queens and resulted in 100% mortality on both substrates. Talstar and Phantom provided significantly lower levels of control relative to Termidor. Talstar

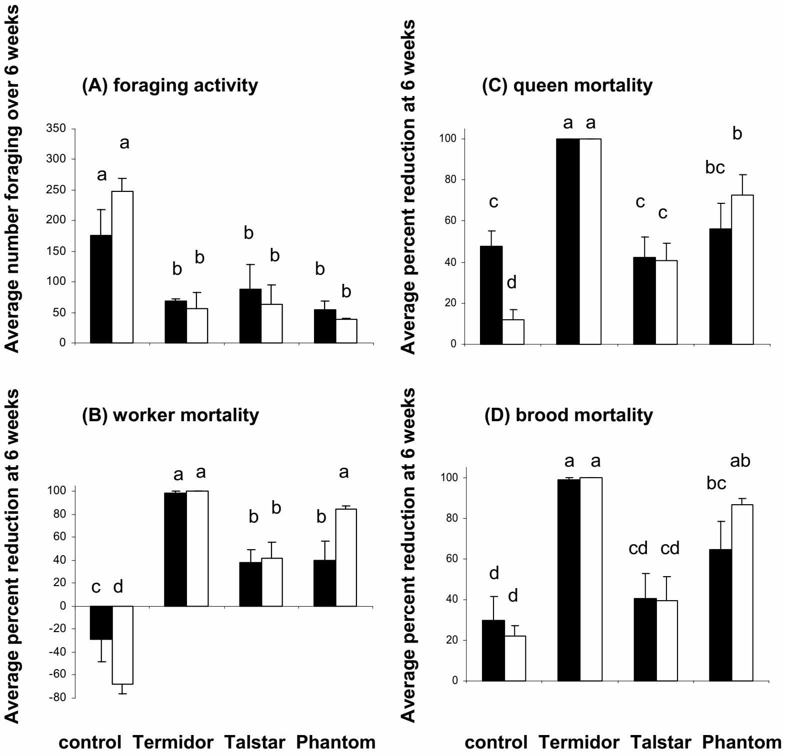

Fig. 3. Impacts of various treatments on colony attributes on outdoor substrates. Solid bars represent concrete pavers; open bars represent hardwood mulch. (A) Cumulative foraging activity over the course of the entire 6-wk study. (B) Cumulative worker mortality over the course of the study. (C) Queen numbers at the end of 6 wk. (D) Brood quantity at the end of 6 wk. Means with the same letter are not significantly different by the LSD *t*-test ($P \leq 0.05$).

queen mortality on concrete was similar to concrete controls, suggesting no efficacy of Talstar against queens. On indoor substrates, Phantom and Demon provided moderate reduction in queen numbers (≈40%). Cinnamite was ineffective on either vinyl or ceramic tile and resulted in negligible queen mortality.

Brood Mortality. No significant substrate effects were observed in association with brood reduction on outdoor substrates. Both Termidor and Phantom were highly effective at reducing the number of brood present. Talstar had no significant effect on brood reduction relative to controls. On indoor substrates, Phantom was the most effective insecticide, providing 45% brood reduction on vinyl tile and 83% reduction on ceramic tile. The efficacy of Cinnamite and Demon was not different from the controls.

Effect of Substrate on Insecticide Performance. Numerous biotic and abiotic factors affect insecticide performance, with the application substrate being one of the most important factors (Wagner and Strawn 1980, Chadwick 1985, Su and Scheffrahn 1990). Our results revealed significant substrate effects, with the nonporous ceramic tile causing faster and greater mortality relative to the porous substrates (mulch, concrete, and vinyl tile). We did not, however, observe a significant difference in insecticide performance between concrete and mulch. Two insecticides, Termidor and Talstar, performed equally well in mulch and on concrete. The impact of Phantom on worker mortality, queen number, and brood quantity was slightly, although not always significantly, higher in mulch. The effect of substrate was more pronounced with vinyl and ceramic tile, with all three insecticides tested generally performing better on ceramic tile. This was most likely due to the nonabsorptive nature of this material. Phantom was the only insecticide tested on all four substrates, thus allowing a comparison between outdoor and indoor materials. Phantom generally performed better on outdoor substrates (Fig. 3). This result is rather unexpected given that concrete and mulch are highly absorptive. However, nest relocation behaviors might provide an explanation for this result. Pharaoh ants moved into the mulch in three of three replicates, two-thirds of concrete replicates, one-third of vinyl tile replicates, and zero of three ceramic tile replicates. Higher incidence of nesting in treated outdoor substrates most likely promoted mortality by increasing proximity to the insecticides. Ants could have become contaminated with insecticide residues in the process of exploring alternative nesting locations and colonizing treated substrates. Furthermore, after colonizing the treated substrates, workers were readily observed exploring the top surface of mulch and concrete. Such behaviors may have con-

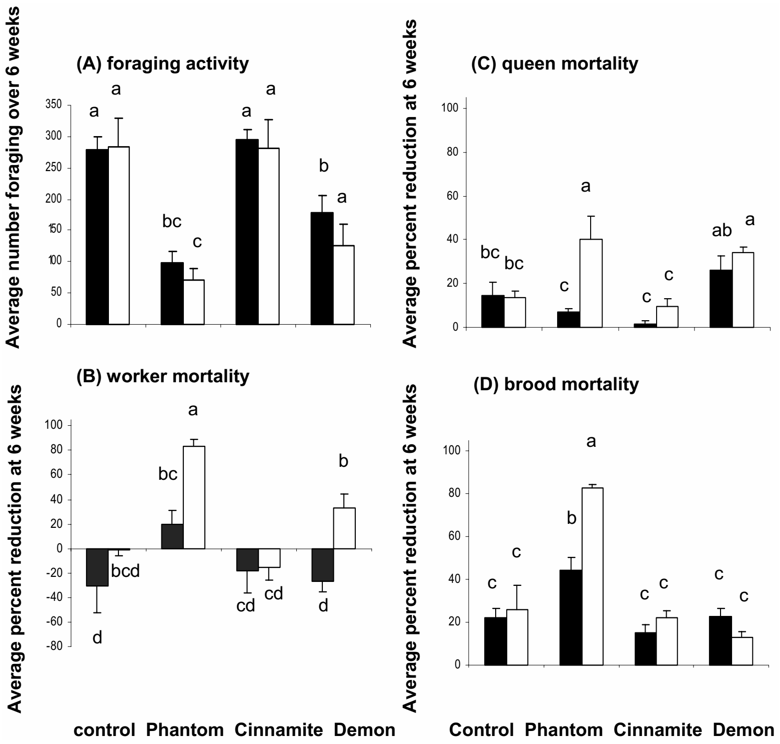

Fig. 4. Impacts of various treatments on colony attributes on indoor substrates. Solid bars represent vinyl tile, open bars represent ceramic tile. (A) Cumulative foraging activity over the course of the entire 6-wk study. (B) Cumulative worker mortality over the course of the study. (C) Queen numbers at the end of 6 wk. (D) Brood quantity at the end of 6 wk. Means with the same letter are not significantly different by the LSD *t*-test ($P \leq 0.05$).

tributed to the higher mortality associated with outdoor substrates.

In conclusion, residual insecticide barriers are commonly used to control various species of house invading ants (Vail and Bailey 2002, Scharf et al. 2004), including Pharaoh ants (Edwards 1986, Oi et al. 1996). They are generally applied outdoors as barrier sprays designed to kill and/or repel foraging ants. Our results demonstrate that nonrepellent residual insecticides, most notably fipronil, can be an effective tool for controlling Pharaoh ants indoors. The Pharaoh ant is primarily an indoor pest, although it also may nest and/or forage outside in warmer climates (Kohn and Vlček 1986, Oi et al. 1994, Vail and Williams 1994). As a result, treatments for Pharaoh ants are usually performed indoors, although outdoor treatments with baits are also effective (Oi et al. 1994, Vail et al. 1996). In the past, residual insecticide sprays have been regarded as inferior to baits and ineffective at eradicating infestations. Here, we show that nonrepellent residual insecticides, which are active at low doses (i.e., fipronil, and to a lesser degree chlorfenapyr) can result in a complete eradication of colonies. Furthermore, our results indicate that residual sprays should be applied to nonporous materials to maximize efficacy. To maximize control efforts in complex field situations (numerous nesting sites in large buildings), a combination of baits and residual sprays might be used, with fipronil being a good candidate. Fipronil is highly effective against Pharaoh ants in bait (Lee 2000) and spray (current study) formulations. Combining both approaches might provide effective Pharaoh ant control under field conditions where numerous colonies may exist.

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