Perimeter Treatments with Two Bait Formulations of Pyriproxyfen for Control of Pharaoh Ants (Hymenoptera: Formicidae)

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ABSTRACT Pyriproxyfen, a juvenile hormone analog, was formulated into 2 types of baits and applied to the exterior of buildings for control of natural infestations of the Pharaoh ant, *Monomorium pharaonis* (L.). Pyriproxyfen baits were formulated at 0.5 and 1% by weight in an unprocessed peanut oil attractant applied to a pregel defatted corn grit carrier and at 0.25% in a composite bait consisting of unknown ingredients. Both baits effectively controlled the ants. Worker numbers were reduced between 84.5 and 94.3% at 2.5 wk after the 2nd bait application when the 0.25% pyriproxyfen composite bait was applied to apartment buildings with separate apartments. Outdoor worker numbers were reduced 84% 2 wk after the 2nd bait application when 0.5% pyriproxyfen bait was applied to a large building with separate wings. A reduction of >85% in worker number was not observed in the 1% pyriproxyfen treatment until 8 wk after the 2nd bait application; however, no ants were ever found indoors in this treatment. Dyes in the pyriproxyfen oil treatments were used to determine if ants foraged from one treatment area to the next. Calco oil dyes used as markers in the oil bait were detectable in the postpharyngeal gland of the queens and workers for up to 14 wk after baiting in the field and 17 wk after baiting in the laboratory. A perimeter treatment applied to large buildings with separate wings was more difficult to evaluate than treatments applied to separate buildings containing multiple apartments because contaminated ants from treated areas, as indicated by the presence of dye, were found in the controls.

KEY WORDS *Monomorium pharaonis,* insect growth regulator, perimeter baiting, marker, pyriproxyfen

PYRIPROXYFEN, A JUVENILE hormone analog (2-[1-methyl-2(4-phenoxyphenoxy) ethoxy] pyridine [McLaughlin Gormley King, Minneapolis, MN]) has been evaluated against urban insect pests including cockroaches (Kawada et al. 1989; Ross and Cochran 1990, 1991; Koehler and Patterson 1991), fleas (Palma and Meola 1990), termites (Su and Scheffrahn 1989), and pest ants (Glancey et al. 1990; Banks and Lofgren 1991; Reimer et al. 1991; Vail and Williams 1995). In most cases, it was an effective control agent. Shifts in caste (Su and Scheffrahn 1989; Glancey et al. 1990; Banks and Lofgren 1991; Vail and Williams 1995), mortality of immatures (Glancey et al. 1990; Banks and Lofgren 1991; Vail and Williams 1995), cessation of or decrease in oviposition (Glancey et al. 1990; Banks and Lofgren 1991; Reimer et al. 1991; Vail and Williams 1995) and toxicity to adults (Vail and Williams 1995) were reported in social insects exposed to pyriproxyfen in baits.

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Pharaoh ants, *Monomorium pharaonis* (L.), are ubiquitous urban pests found indoors and outdoors in warmer climates but restricted to indoor occupation in cooler climates (Edwards 1986). Baits containing insect growth regulators, such as methoprene and fenoxycarb, also have provided control of Pharaoh ants (Wilson and Booth 1981; Edwards 1985; Williams 1990; Williams and Vail 1993, 1994). Pyriproxyfen bait also has been effective in controlling Pharaoh ants in laboratory tests (Vail and Williams 1995). Despite their efficacy, no insect growth regulators are currently commercially available for Pharaoh ant control in the United States.

When Pharaoh ants forage outdoors, placing baits where ants are actively foraging on the outside of structures reduces application time and exposure of residents to insecticides (Oi et al. 1994). Placement of baits containing metabolic inhibitors solely around the outside perimeter of structures has controlled Pharaoh ants effectively in Texas (Haack 1991) and Florida (Oi et al. 1994, 1996).

Our objectives were to evaluate a peanut oil on corn grit formulation of pyriproxyfen for Pharaoh ant control when placed exclusively around the outside perimeter of a large structure, and to evaluate a composite bait formulation of pyriproxyfen when placed around the outside perimeter and inside of apartment buildings. Also, laboratory colonies were subjected to the same pyriproxyfen oil baits to evaluate the efficacy of the material applied in the field.

Materials and Methods

Peanut Oil on Corn Grit Formulation. Two concentrations (0.5 and 1%) of pyriproxyfen were formulated by weight in unprocessed peanut oil. Calco red and blue dyes (2% by weight, Pylam Products, Garden City, NY) were added to the 0.5 and 1% solutions, respectively, to act as markers. Dyes were used to determine if ants were foraging from one treatment area to the next. This pyriproxyfen–oil–dye solution was applied to a pregel defatted corn grit carrier (Illinois Cereal Mills, Paris, IL) to compose 30% of the bait by weight; a control bait was formulated using unprocessed peanut oil. Each treatment was placed in a separate bait station using 1 g of 1% pyriproxyfen and the untreated control bait and 2 g of 0.5% pyriproxyfen bait. The bait stations consisted of a petri dish (50 by 9 mm) with 9 holes in the lid that allowed Pharaoh ant entry and prevented bait from falling out. In addition, the top and bottom of the petri dish were sealed together with acetone to seal the dish and prevent bait spillage.

Field (Large Structure). A natural infestation of Pharaoh ants located at the Jacksonville Naval Air Station's Bachelor Officer's Quarters, Jacksonville, FL, was used for this trial. This 3-story building consisted of 3 wings with a central area connecting all wings. Each room of the building opened onto an outdoor concrete walkway. The outside wall of each room consisted of 2–3 glass windows reaching from ceiling to floor supported by aluminum framing. Most rooms had a sink area, bedroom, and bathroom with shower and toilet (total floor area ≈35 m^2).

On 26 September 1991, initial Pharaoh ant population estimates were determined by placing index cards containing ≈1 g of peanut butter at various locations throughout the building. After 2 h, ants on each card were counted and the cards removed. Then the east and west wings were treated with 84 of the 1 and 0.5% pyriproxyfen baits, respectively; the south wing and central area received 92 control baits. Two bait stations were placed on the outside of each room. Usually, 1 bait station was placed on the base of the middle window frame and 1 on the stucco that separated the rooms. Baits also were placed in the 2 end hallways surrounding the east and west wing to provide any ants foraging in these wings the opportunity to feed upon bait.

Baits were applied once per week for 2 wk and removed 1 wk after application. Pharaoh ant outdoor populations were monitored using index cards containing 1 g of peanut butter placed in the bait station locations 1 wk later and every 2 wk thereafter until the 18th wk. In addition to outdoor monitoring, indoor monitoring was initiated 10 wk after baiting coinciding with the onset of cooler temperatures and low outdoor ant counts. Four corner rooms per floor per wing were used. Monitoring cards were placed in 6 locations: on the floor of the bathroom by the toilet, on the counter by the bathroom sink, on the floor by both sides of the bed, and on the floor by the door and window. The cards were left in place for 2–3 h, after which ant species and number of individuals were recorded. Cards with ants were placed in a self-sealing plastic bag, taken to the laboratory, and the ants were crushed on a white piece of paper with a heavy metal roller to determine the presence of dye.

Descriptive statistics (mean and standard error) of the number of Pharaoh ants per card and percentage of dyed Pharaoh ants were calculated per room for each treatment.

Laboratory. Previously described baits and bait stations were placed in laboratory colonies to evaluate their efficacy. Two bait stations were provided to each of 3 colonies and left in place for 1 wk, at which time they were replaced with fresh bait and again removed after 1 wk. Following bait treatments, each colony was provided with food cups containing house flies, hard-boiled chicken egg, and honey agar twice a week. Worker number, queen number, and brood rating were recorded once a week after the initial bait application. Brood (eggs, larvae, and pupae) was rated by visually comparing photographs of known quantities of brood with the brood in a cell (Vail and Williams 1995).

Brood rating and worker and queen numbers were analyzed using general linear models and Ryan–Einot–Gabriel–Welsch Q test (SAS Institute 1993) to determine differences in treatments.

Composite Bait Formulation, Field Apartments. A natural infestation of Pharaoh ants was located at an apartment complex in Gainesville, FL. All routine pest control services to these apartments were suspended for the duration of the study. Six single-story buildings consisting of 4 single or double bedroom apartments were used. Initial populations of Pharaoh ants were estimated by placing index cards containing ≈1 g peanut butter at 6 locations inside as well as outside each apartment. Indoor card locations were the living room window, kitchen sink and fuse box, bathroom basin and toilet base (or an interior corner of the shower if pets or small children were present), and a bedroom window. Outdoor card locations were above ground level to prevent competition by the red imported fire ant, *Solenopsis invicta* Buren (Oi et al. 1994) and were the base of the front door frame, courtyard gate and window, and top of the intersection of the courtyard and apartment wall. Other outdoor card locations included the opposite side of the courtyard door, water spigots, electrical or telephone utility boxes, junction of the air conditioner and wall, and cracks where ants were seen

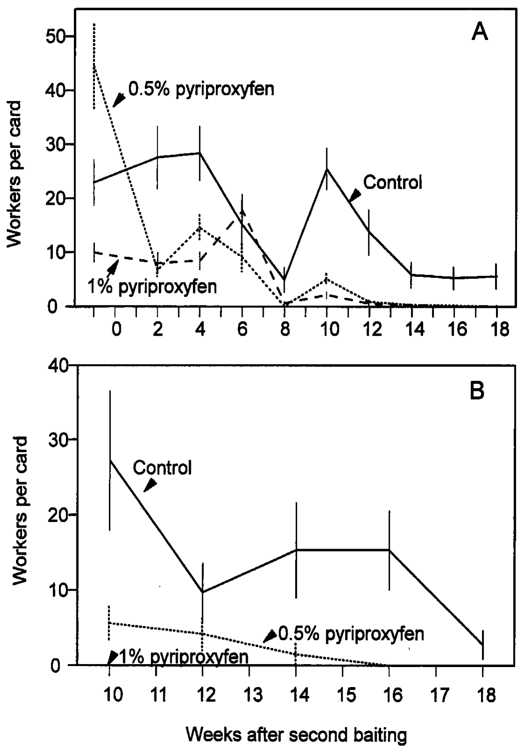

Fig. 1. Mean number of Pharaoh ant workers per monitoring card placed (A) outside or (B) inside of the Jacksonville Naval Air Station BOQ following treatment with unprocessed peanut oil baits containing pyriproxyfen. No ants were found inside rooms treated with 1% pyriproxyfen. Vertical lines indicate 1 SEM.

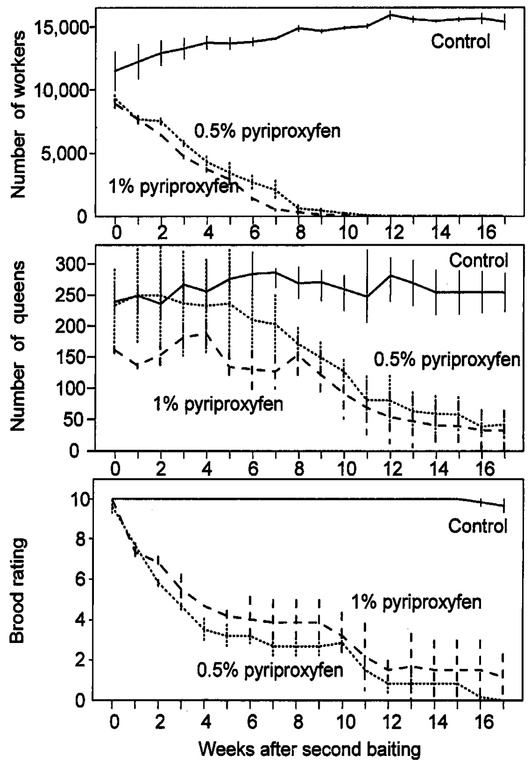

Fig. 2. Mean worker number, queen number, and brood rating of Pharaoh ant colonies exposed to unprocessed peanut oil baits containing pyriproxyfen in the laboratory. Vertical lines indicate 1 SEM.

foraging. Outdoor cards were adhered with a reusable adhesive (Fun-Tak, Dap, Dayton, OH) between 0900 and 1130 hours EDST. Cards were checked 2 h after the initial placement and the number of ants per card was recorded. Ants were tapped back onto the structure.

Pyriproxyfen (0.25%) was formulated in a proprietary combination attractant and carrier bait; the control bait consisted of pregel defatted corn grit only. Three buildings each were chosen randomly to receive the pyriproxyfen and control bait treatments. Two grams of bait were placed in the same stations as previously described. Bait applications were made once per week for 2 wk and removed 1 wk after application. Bait stations were placed next to locations used for the initial card survey. Before the card was removed, ants were counted and then tapped onto the adjacent bait station. Pharaoh ant populations were monitored as described in the initial survey at 2.5, 6, 12, 16, and 20 wk after the 2nd bait application. Mean temperatures during monitoring periods ranged from 27.5 ± 3.7°C (mean ± SD) during placement of the cards and 31.3 ± 4.1°C during retrieval of the cards. This study was conducted between 11 June and 5 November 1993.

Mean percentage reduction in Pharaoh ants per card per building based on the initial survey was evaluated for each sample date. Also, the percentage of the monitoring cards with ants was calculated per building. All percentage data were subjected to arcsine transformation before analysis of variance (ANOVA) (SAS Institute 1993).

Results

Peanut Oil on Corn Grit Formulation. *Field (Large Structure).* More workers per card were found in the control wing than in the treated wings on every date except at week 6 after the 2nd bait application and at week 0 (Fig. 1). After week 8, a mean ant count of >5 per card in baited areas was found only once, during week 10. The number of workers per card was reduced by ≈84% by 2 wk after the 2nd bait application in the 0.5% pyriproxyfen treatment. Workers per card were not reduced by 85% until 8 wk after the 2nd application in the 1% pyriproxyfen treatment. No ants were found indoors in the wing treated at 1% during any week or in the wing treated at 0.5% after week 16, although ants were found in the control areas during the entire study (Fig. 1).

Laboratory. Worker numbers gradually declined (Fig. 2). At 9 wk, the mean worker number for the

Table 1. Mean percentage ant reductions and cards with ants in indoor and outdoor monitoring sites at an apartment complex following treatment with 0.25% pyriproxyfen in a composite bait, Gainesville, FL, 11 June–5 Nov. 1993

Treatment	Mean no. ants per cards ± SE	Weeks after 2nd treatment				
		2.5	6	12	16	20
				Mean ± SE % reduction in ants per card		
0.25% pyriproxyfen	26.6 ± 9.3	85.5 ± 2.2	95.6 ± 4.0	99.9 ± 0.1	100.0 ± 0.0	100.0 ± 0.0
Control	13.5 ± 8.4	4.9 ± 4.9	51.7 ± 25.9	18.6 ± 18.6	9.2 ± 9.2	16.3 ± 8.7
P	0.3553	0.0014	0.1350	0.0114	0.0017	0.0006
				Mean ± SE % monitoring cards with ants		
0.25% pyriproxyfen	35.9 ± 10.7	30.1 ± 10.6	13.3 ± 7.3	2.8 ± 1.5	0.0 ± 0.0	0.0 ± 0.0
Control	30.9 ± 11.2	27.2 ± 8.0	15.9 ± 5.3	28.5 ± 8.7	19.4 ± 2.8	24.0 ± 3.7
P	0.7766	0.8649	0.6364	0.0303	0.0002	0.0003

control colonies was 14,667 compared with 442 and 115 in the colonies treated with 0.5 and 1% pyriproxyfen, respectively. One week after the 2nd baiting and each week thereafter, significantly fewer workers were recorded in the treated colonies than in the control colonies (week 2; $F = 10.04$; df = 2, 6; $P = 0.0122$).

These laboratory tests simulated the reductions found in the field trials. In the laboratory, 7 and 8 wk were required to reduce worker populations by >85% for the colonies treated with 1 and 0.5% pyriproxyfen, respectively. In the large structure, both concentrations reduced worker number by 85% by week 8; although 84% reduction in worker number had been achieved in the 0.5% treatment by week 2, it did not remain so (Fig. 1).

The number of queens declined slowly in the treated colonies and were not significantly lower than in the control until week 9 ($F = 6.35$; df = 2, 6; $P = 0.033$; Fig. 2). Queens in treated colonies were observed wandering the trays starting around week 4. The decline in queen number could be attributed to death by old age, starvation from lack of worker attention, or toxic effects caused by the accumulation of pyriproxyfen.

The amount of brood showed a rapid decline following treatment (Fig. 2). Brood rating was significantly lower in the treated colonies than in the control colony for every week beginning 1 wk after the 2nd baiting (Fig. 2; $F = 114.0$; df = 2, 6; $P = 0.0001$), demonstrating that pyriproxyfen prevents development of immature workers.

Both the red and blue dye were apparent in colonies 1 wk after the 1st bait treatment. Dyes were visible in the postpharyngeal gland of both queens and workers and in the crop of workers. Dye also was visible in most larvae, indicating that pyriproxyfen was well distributed throughout the colonies. Dye was not found in any pupae.

Composite Bait. Ant number, determined by combining counts from the indoor and outdoor cards, was significantly reduced by 85.5% ($F = 61.14$; df = 1, 4; $P = 0.0014$) 2.5 wk after treatment in the apartments receiving the pyriproxyfen composite bait (Table 1). Mean ant number in the pyriproxyfen treatment gradually decreased from 2.5 to 16 wk, when total elimination occurred. The percentage of monitoring cards with ants decreased slowly and was not significantly lower than the control until 12 wk ($F = 10.8$; df = 1, 4; $P = 0.0303$) after treatment. No ants were found on any cards in the pyriproxyfen-treated buildings at 16 or 20 wk (Table 1).

More ants were found outdoors than indoors as evidenced by the mean number of ants per card (Tables 2 and 3) and the percentage of cards with ants (Tables 2 and 3) for the pretreatment counts. Outdoors, the number of ants per card was significantly reduced by 84.5% ($F = 752.2$; df = 1, 4; $P = 0.0001$) by the 1st sample date after treatment and followed the same trends as the indoor and outdoor data combined. Complete elimination did not occur until 16 wk after treatment (Table 3).

Table 2. Mean percentage ant reductions and cards with ants in indoor monitoring sites at an apartment complex following treatment with 0.25% pyriproxyfen in a composite bait, Gainesville, FL, 11 June–5 Nov. 1993

Treatment	Mean no. ants per card ± SE	Weeks after 2nd treatment				
		2.5	6	12	16	20
				Mean ± SE % reduction in ants per card		
0.25% pyriproxyfen	5.9 ± 3.7	94.3 ± 4.3	99.0 ± 1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Control	14.1 ± 11.4	33.3 ± 33.3	100.0 ± 0.0	11.5 ± 11.5	38.8 ± 30.9	61.1 ± 30.9
P	0.5345	0.1831	0.3739	0.0029	0.1255	0.2303
				Mean ± SE % monitoring cards with ants		
0.25% pyriproxyfen	17.1 ± 11.5	11.1 ± 8.5	3.7 ± 3.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Control	13.9 ± 7.3	11.1 ± 5.6	0.0 ± 0.0	13.9 ± 2.8	16.7 ± 12.7	13.9 ± 10.0
P	0.9104	0.9438	0.3739	0.0009	0.1786	0.1637

Table 3. Mean percentage ant reductions and cards with ants in outdoor monitoring sites at an apartment complex following treatment with 0.25% pyriproxyfen in a composite bait, Gainesville, FL, 11 June–5 Nov. 1993

Treatment	Mean no. ants per card ± SE	Weeks after 2nd treatment				
		2.5	6	12	16	20
		Mean ± SE % reduction in ants per card				
0.25% pyriproxyfen	39.2 ± 13.7	84.5 ± 3.0	92.6 ± 7.0	99.7 ± 0.1	100.0 ± 0.0	100.0 ± 0.0
Control	14.8 ± 11.0	0.0 ± 0.0	31.8 ± 19.8	18.6 ± 18.6	5.3 ± 5.3	5.6 ± 4.2
P	0.2378	0.0001	0.0512	0.0120	0.0005	0.0002
		Mean ± SE % monitoring cards with ants				
0.25% pyriproxyfen	54.6 ± 14.4	47.7 ± 13.4	22.1 ± 11.5	4.6 ± 2.5	0.0 ± 0.0	0.0 ± 0.0
Control	34.7 ± 14.1	35.3 ± 13.8	22.3 ± 6.7	36.1 ± 11.8	20.8 ± 4.8	29.5 ± 4.0
P	0.3653	0.5575	0.7633	0.0486	0.0015	0.0002

Reduction in mean ant number occurred more rapidly indoors where ants were less abundant before and after treatment (Table 2). Reduction of 94.3 and 100% occurred by 2.5 and 12 wk after treatment, respectively. Mean percentage of cards with ants decreased gradually in the pyriproxyfen-treated buildings from 11.1% for week 2.5 to 0% by week 12 (Table 2). Indoors, the pyriproxyfen treatment was significantly different from the controls only at 12 wk after treatment for both variables (mean number ants per card [$F = 42.1$; df = 1, 4; $P = 0.0029$] and percentage of cards with ants [$F = 78.9$; df = 1, 4; $P = 0.0009$]).

Discussion

A large building with separate wings is not an ideal structure for evaluating baits for Pharaoh ant control. The maximum and mean dispersal distances determined for Pharaoh ants in a large structure were 45 and 16.2 m, respectively (Vail and Williams 1994). Even though Pharaoh ants travel long distances and trophallaxis occurs between neighboring colonies (Edwards 1986), it is unlikely that ants at one end of a large structure can communicate with ants at another end. However, determination of the degree of communication between ant populations within a large structure is essential for evaluation of field data. Dyes also should be used to determine whether ants are foraging from one wing to the next.

Table 4. Mean percentage of Pharaoh ant foragers containing dye in pyriproxyfen treatments from large-building field trial, Jacksonville Naval Air Station

Weeks[a]	Mean ± SD % foragers with dye		
	Control	0.5%[b]	1%[b]
2	11.3 ± 23.77	97.8 ± 3.52	84.0 ± 14.35
4	2.7 ± 7.56	52.2 ± 29.11	91.1 ± 14.21
6	7.3 ± 25.69	23.9 ± 27.78	90.6 ± 9.12
8	11.1 ± 33.33	0	65.4 ± 33.48
10	0	0	22.6 ± 35.20
12	0	0	12.9 ± 35.22
14	0.03 ± 0.13	0	18.7 ± 28.06
16	0	0	—[c]

[a] Weeks after 2nd treatment on 3 Oct. 1991.
[b] Percentage pyriproxyfen by weight.
[c] No ants found.

Dyes in baits can be used to determine if ants are foraging from one treatment area to the next at a potential field trial site. Calco blue dye was an excellent marker to detect the presence of the pyriproxyfen–oil bait in the ants throughout the structure. This dye was detected in the foraging population in the field for at least 14 wk after baiting. The blue dye also was detected in the postpharyngeal glands of the queens and workers for at least 17 wk after baiting under laboratory conditions. Areas where contamination is detected can be excluded from evaluation, if possible.

In our large-building field trial, dye was well distributed (>80%) throughout foragers in both treated wings 2 wk after the 2nd applications of insect growth regulator (Table 4). However, 11% of the ants collected in the control wing contained dye 2 wk after bait application (Table 4). Therefore, ants living in the control wing were contaminated with the insect growth regulator, although not to the same extent as the ants in the treated wings. The dye data indicate we could not guarantee that each room where our large-building trial was conducted represented a separate population. As a result, descriptive statistics were used with this study to prevent violating the assumption of independent samples used in ANOVA.

Pyriproxyfen baits formulated at 0.5 and 1% by weight in unprocessed peanut oil on a pregel defatted corn grit carrier reduced Pharaoh ant populations in the large-building field trial (Fig. 1). Eight weeks after bait application, most workers in the pyriproxyfen treatments not affected directly by insect growth regulator toxicity were probably dying because they reached the end of their estimated maximum life span of 9–10 wk (Peacock and Baxter 1950). This time frame to reduction of worker numbers corresponded with results of our laboratory work (Fig. 2). In addition to bait trophallaxis contamination of the control wing, the large-building field trial could have been affected by removal of ants from the structure to determine dye longevity or by reduced fall and winter temperatures. Morning temperatures for sample dates on weeks 10, 12, 14, 16, and 18 were 21.5, 16.5, 11.5, 20.5, and 15°C, respectively. The mean number of Pharaoh ants per card for the controls on week 10 was 25; however, the number per card was much lower (5.2) on week 16 when the temperature was similar to that of week 10. Therefore, temperature alone did not explain the decrease in ant abundance in the controls.

Despite the inconclusive results, application of the unprocessed peanut oil on pregel defatted corn grits baits to the exterior of the building was effective (Fig. 1), although the placement of the bait outdoors in the large-building trial could have been more selective. Baits were placed approximately the same distance apart throughout the building, and baits placed on the 1st floor often were consumed by fire ants. Placing baits above ground where monitoring has indicated an infestation, or where Pharaoh ants are currently foraging, may increase the likelihood that Pharaoh ants are the 1st foragers to reach the bait (Oi et al. 1994, 1996).

In the field evaluation of the 0.25% pyriproxyfen composite bait, average reductions in worker number were between 84.5 and 94.3% after only 2.5 wk and increased through time. This rapid reduction in worker number is similar to results with baits containing metabolic inhibitors (hydramethylnon and sulfluramid) applied at the same apartment complex in an earlier study (92.6–100% reduction in ant counts by wk 1) (Oi et al. 1994). However, worker number increased 4 wk after treatment with use of a metabolic inhibitor (Oi et al. 1994). In contrast, the pyriproxyfen composite bait completely reduced worker number between 12 and 16 wk with no reinfestation occurring through termination of the study (20 wk).

Pyriproxyfen is an effective insect growth regulator for controlling natural infestations of Pharaoh ants and may provide more long-term control of Pharaoh ants. Insect growth regulators were spread more extensively through neighboring colonies than metabolic inhibitors in laboratory evaluations (unpublished data). Metabolic inhibitors act more quickly, eliminating foragers before they can spread the toxicant to neighboring colonies. In a field study comparing an insect growth regulator (fenoxycarb) with a metabolic inhibitor (sulfluramid), fenoxycarb provided longer Pharaoh ant control (Williams and Vail 1994). Fenoxycarb baits (0.5%) in peanut oil completely eliminated Pharaoh ants for 18 wk (weeks 6–24), whereas Pharaoh ants were detected in rooms receiving sulfluramid bait 6 wk after ants were considered eliminated. Pharaoh ants may not be eliminated from a structure using a metabolic inhibitor if all colonies in a site are not located or if baits are not placed close enough to the nest site or foraging trails. Application of an insect growth regulator bait 1 wk before a metabolic inhibitor bait may provide both rapid kill and long-term control.

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