Fipronil Trials in California Against the Red Imported Fire Ant, *Solenopsis invicta* Buren, Using Sugar Water Consumption and Mound Counts as Measures of Ant Abundance[1]

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J. Agric. Urban Entomol. 20(4): 221–233 (October 2003)

ABSTRACT Using sugar water consumption and mound counts, we evaluated the effectiveness of fipronil and other toxicants against red imported fire ants, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), around residences and at a golf course fairway at two California country clubs. We counted the number of mounds and measured sugar water consumption at bait stations adjacent to the homes. We compared three formulations of granular fipronil with a bait treatment consisting of pyriproxyfen followed one week later by hydramethylnon. One month after treatment, the bait treatments near the residences showed a significantly greater reduction in ants than the combined fipronil treatments for both number of mounds (reductions of 91% versus 73%) and sugar water consumption (reductions of 91% versus 81%). Fifteen months after treatment, mound reductions for the bait and fipronil treatments were 79% versus 92% ($P = 0.06$), respectively, and reductions in sugar water consumption were 95% versus 99% ($P = 0.03$), respectively. Decreases in ant abundance, as measured by sugar water consumption and mound counts, were significantly correlated during the experiment ($r_s = 0.68$). On the golf course fairway, we used sugar water consumption to compare one treatment of pyriproxyfen with one of fipronil. Within one month, the fipronil treatment had reduced sugar water consumption by 68%, whereas the pyriproxyfen reduction was 33%. However, after nine months the respective reductions were similar at 98% and 97%. Overall, on the fairway, the posttreatment results of the fipronil were significantly better than the pyriproxyfen results ($P = 0.01$).

KEY WORDS *Solenopsis invicta*, fipronil, Hymenoptera, fire ant, ant monitors, pyriproxyfen, hydramethylnon

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The red imported fire ant (RIFA), *Solenopsis invicta* Buren (Hymenoptera: Formicidae), was detected in California in 1997 in almond groves in Kern County in honeybee colonies from Texas (Dowell et al. 1997). These outbreaks were believed to be restricted to the immediate vicinity of the groves. In 1998, a much more significant outbreak was detected in plant nurseries, golf courses, and surrounding neighborhoods of Orange County and the Coachella Valley of Riverside

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[1] Accepted for publication 25 May 2004.

[2] For correspondence and reprints

County (California Department of Food and Agriculture 1998, Greenberg et al. 1999, Jetter et al. 2002). These discoveries led to a quarantine of all of Orange County and of portions of the Coachella Valley. The high ant density at these locations suggests that fire ants could have been undetected for several years.

In response to the RIFA invasion, a large-scale eradication program began in southern California in 1999 involving several organizations: the California Department of Food and Agriculture, Vector Control of Orange and Riverside Counties, and the Agricultural Commissioner's Offices of Orange, San Diego, Riverside, Los Angeles, and San Bernardino counties (California Department of Food and Agriculture 1999). In 1999 we began field studies of RIFA in the Coachella Valley to help the eradication effort evaluate new and existing pesticide treatments.

Fipronil (BASF, Research Triangle Park, North Carolina) is a relatively new class of pyrazole insecticide (Colliot et al. 1992) that is an antagonist of γ-aminobutyric acid, an inhibitory neurotransmitter (Durham et al. 2001), that has been introduced for the control of *S. invicta*. It received US Environmental Protection Agency approval for use on fire ants in 2001. In trials in the southeastern United States, fipronil has been shown effective against RIFA (Collins & Callcott 1998, Sparks & Diffie 1998, Collins et al. 1999). In Texas, 2.3 g of AI/ha (0.0125 lb AI/a) fipronil provided 95% reduction in mounds from weeks 12 through 52 after application (Barr & Best 2003). Fipronil would assist the California RIFA eradication program if a single treatment could substitute for two or more treatments of other products. Furthermore, its application does not require turning off the irrigation, a major hardship in the hot, desert climate of the Coachella Valley.

Fipronil has previously been tested in the southeastern United States, where rainfall is adequate for RIFA survival, but most of southern California receives approximately 25.4 cm of rain concentrated during a winter rainy season from November to March. Therefore, the fire ants live here only with abundant irrigation. We wanted to know whether fipronil worked as well under these conditions.

Materials and Methods

Although fire ant mound counts are frequently used elsewhere to evaluate pesticide treatment effects, some of our plots have no visible mounds (e.g., fairways in golf courses, irrigated strips next to sidewalks, under weed guard in plant nurseries). For these situations, we wanted an additional measure of ant abundance. Besides mound counts, other measures of ant abundance have been tried. For RIFA-detection surveys, the California Department of Food and Agriculture has been using one-inch cubes of luncheon meat placed on the ground in small, plastic lures designed for Medfly detection. Drees (1994) placed olive oil–soaked index cards (2.54-cm square) along a transect on an island off the coast of Texas to measure RIFA density. We decided to evaluate sugar water consumption at monitoring stations as a measure of RIFA abundance in California. For Argentine ants, sugar water has already proven useful for monitoring foraging activity and evaluating chemical treatments (Reierson et al. 1998, Klotz et al. 2000, Costa et al. 2001). Leaving these monitors in place for 24 h assures that the ants can visit them during their peak foraging times. Consumption is proportional to the number of ant visits and is therefore an indirect measure of ant abundance. Of course, food preferences can change throughout time, so we are measuring a change in foraging activity with respect to other treatments in the same study.

We have determined that the average RIFA worker at our test sites consumes 0.33 mg of 25% sugar water (unpublished data). This statistic can be used to translate between consumption of sugar water and the number of ant visits to the sugar water by dividing the consumption in ml by 0.00033. Thus, 1 ml of consumption accounts for roughly 3030 ant visits.

House treatments. We selected houses with fire ant infestations at the Sunrise Country Club, Rancho Mirage, California, for our trials. This location is an irrigated oasis in a desert where summer temperatures frequently reach 43°C. Sprinklers are on several times each day, and the grass is mowed frequently where it is adjacent to the golf course. We surveyed the backyards of houses contiguous with the golf course. Most of the fire ants living in these areas were either in the flowerbeds or in the turf within 6 m of the houses. Collection of ants from this location showed the presence of multiple inseminated queens in the colonies. The majority of mounds near the houses were tiny and only several inches in diameter.

Our plots consisted of 6-m wide swaths between the homes and the fairways. At least 15 m separated our treatments from those on the fairways. We selected 18 properties, each about 38 m in length, for our pesticide applications, which were made on 23 August 1999. Moving from one house to the next around the country club, we alternated among three treatment regimes: 2.3 kg/ha (12.5 lb/a) of 0.1%, 16.1 kg/ha (87.4 lb/a) of 0.0143%, or 32.2 kg /ha (174.8 lb/a) of 0.0143% granular fipronil, repeated six times in that order. The first two applications were equivalent to 2.3 g AI/ha (0.0125 lb AI/a), whereas the last was 4.6 g AI/ha (0.025 lbs AI/a). The fipronil was applied with drop spreaders that we calibrated for these application rates. Treatments were applied to the entire backyard of the house and 20 m beyond it on both sides of the house (leaving no area untreated), but no data were collected there because these areas served as unmonitored buffers between treatments. The pesticide was applied with a hand spreader in the adjacent flowerbeds.

The entire 40-ha community, including homes, fairways, and greens, was treated at approximately the same time either by the management or by us. The country club did not repeat their treatments before the end of this study, and we matched our treatments to theirs. For comparison with our plots, we monitored eight houses that the management had treated. They applied 276 g/ha (1.5 lb/a) of 0.5% pyriproxyfen (Distance®, Valent, Walnut Creek, California) during a three-day period starting on 17 August 1999, followed by an application of 276 g/ha (1.5 lbs/a) of 0.73% hydramethylnon (Amdro®, BASF, Research Triangle Park, North Carolina) during a three-day period starting on 1 September 1999. We shall refer to this sequential application of the two baits as the "bait treatments." We used two measures of ant abundance: 1) sugar water consumption over 24 h at bait stations, and 2) mound counts.

Sugar water consumption. Sugar water bait stations (Fig. 1) consisted of 15-ml plastic graduated tubes (Falcon™, Fisher Scientific, Springfield, New Jersey) filled with 10 ml of 25% sugar water (wt/vol). These tubes have 0.5-ml gradations, enabling us to approximate readings to approximately 0.1 ml. These tubes were placed on small wooden logs (#1 whole logs, 1 slot, K'Nex Industries, Hatfield, Pennsylvania) on the ground with the mouth of the tube slightly el-

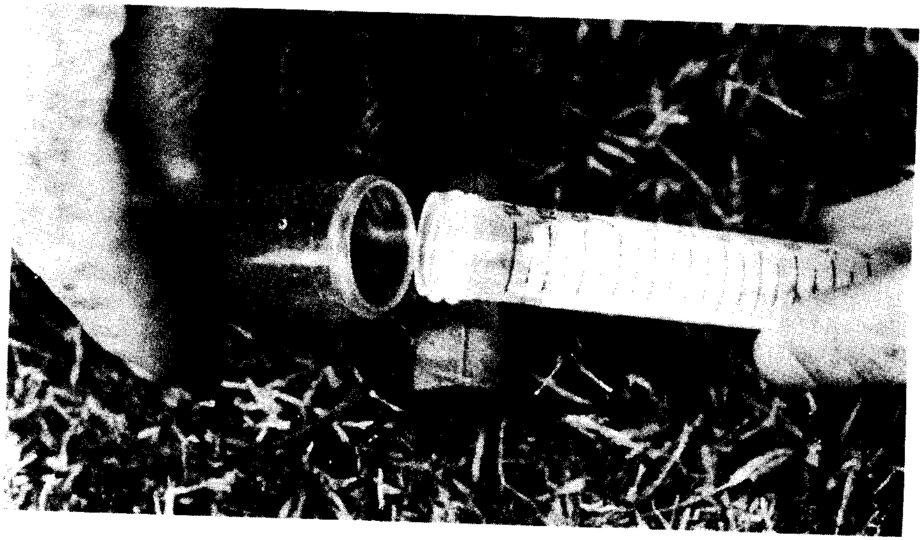

Fig. 1. Vial resting on small toy log on ground. The 15-ml vial on right contains approximately 10 ml of 25% sugar water. The Vial on left is inserted over the mouth of the sugar water vial. See text for details.

evated to prevent spillage. The logs have a convenient groove to hold and elevate the tube to keep it from rolling. Over the mouth of the tube we inserted a 5-dram plastic vial (2.3 × 5.08 cm). It prevented sprinkler water and large insects from entering the tube and reduced evaporation.

We recorded the sugar water volume before placing it on the ground and again 24 h later. We found that, within the limits of our measurement capabilities (0.1 ml), when no ants were present (as was the case for dozens of vials not near mounds during preliminary trials) there was no measurable evaporation, even in the summer. Throughout the experiment, as ant numbers plunged, the most common finding was no change in volume of the monitors. Undoubtedly, high humidity near the ground caused by sprinklers kept evaporation to a minimum.

In treated plots, we placed sugar water bait stations every 6 m along the edge of the turf adjacent to the flowerbeds behind the houses. The monitors were placed at the same location for each sample period, and were usually at least 3 m from the house's wall. As a result of the eradication effort, we could not leave untreated plots, but there was an isolated section of the country club that the landscapers did not treat at the beginning of this study. (This area was on an irrigated slope adjacent to a natural wash area, giving rise to jurisdictional problems and concerns about pesticide runoff.) On this slope, we measured sugar water consumption at the bases of trees. The trees were at least 20 m apart, and each had ants nesting at its base. Mounds were not apparent except at the trees, where the ants were living around the roots and trunks.

Whether at houses or at trees, we considered the monitors to be replicates because each one measured ant abundance in its vicinity and was in the same location for every sample date. As in a Repeated Measures design, they are compared with themselves at future dates and therefore act as their own controls. Although not adjacent to houses, monitors at the trees provided information on seasonal changes in ant sugar water consumption. In the absence of pesticide we expect ant numbers and sugar water consumption at both locations to rise and fall seasonally, and it is these fluctuations that we wish to control with the data from the trees.

Using sugar water consumption, our experimental design allowed us to compare ant abundance over time near homes that received pesticide treatments and untreated areas at the trees. We also compared outcomes among the houses that received either the granular fipronil treatment or the bait treatment. Sampling dates are shown on the x-axis of Fig. 2.

Mound counts. In house backyards that received either the granular fipronil or the bait treatments (the same houses that were monitored above with sugar water), we counted the number of mounds per backyard (the treated area of 6 × 38 m). We compared outcomes among these treatments, but there was no untreated area where we could count mounds. For mound counts, *n* is the number of houses. Sampling dates are shown on the x-axis of Fig. 3.

Fairway treatments. We treated part of a golf course at the Las Palmas Country Club in Rancho Mirage, California. We treated 0.6 ha at one end of this fairway with 2.3 kg/ha (12.5 lbs/a) of 0.1% fipronil; the remaining 0.6 ha received a treatment with pyriproxyfen at 276 g/ha (1.5 lb/a). We did not collect data along a 15-m wide area between the two treatments that was treated but not monitored. Applications were made on August 24, 2000, using drop spreaders that we calibrated for the specific application rate. We monitored 24 trees along the edge of the fairway for ant activity (13 for fipronil and 11 for pyriproxyfen) by measuring sugar water consumption by the ants at the bases of the trees (mounds were not visible on the fairway). Another 36 trees in a section of the country club not yet treated by landscapers (again as a result of water runoff issues) were monitored as controls. Because the eradication program made it impossible to have additional large experimental plots, we considered the trees to be subplots within treatments. The monitors measure ant abundance in their vicinity, and are in the same location for every sample period. Sampling dates are shown on the x-axis of Fig. 4.

Statistics. Before performing statistical tests, we examined the data graphically using probability plots and histograms (Systat 1999). Logarithmic transformations of sugar water consumption ($X' = \ln(X + 1)$) and a square root transformation for the number of mounds ($X' =$ square root $(X + 0.5)$) gave acceptable distributions for analysis. An overall repeated measures analysis on the house data would not be very meaningful because there are different outcomes at different time periods. We therefore combined some of the data and compared their grand means (see below). Outcomes for the fairway treatment were more consistent and we did both an overall repeated measures analysis as well as analyses for some combined dates.

Results

Figures 2–4 show all the sampling dates, whereas Tables 1 and 2 show statistics for both combined periods and some single samples. For statistics on mound counts (see below) we combined four samples in September, two in Octo-

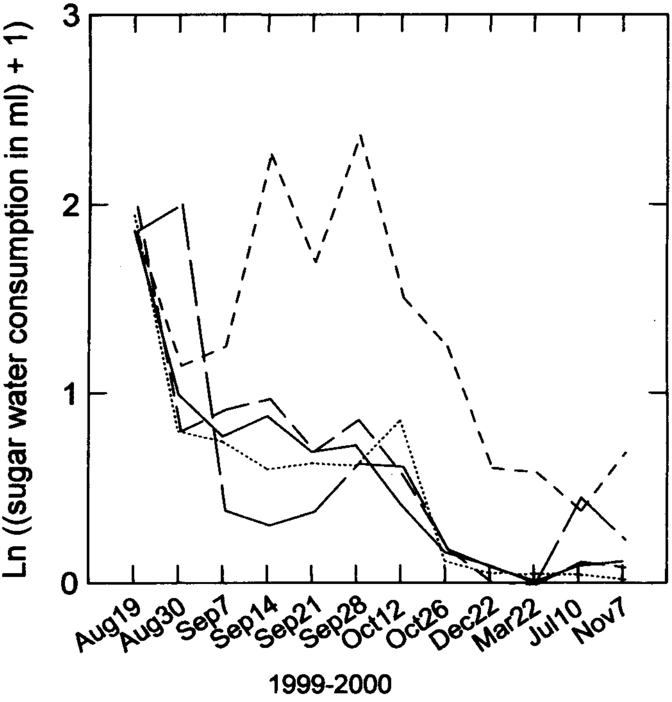

Fig. 2. Natural log of sugar water consumption near homes during 24 h. The x-axis shows the sampling dates.

ber, and three for November through March. For sugar water consumption near homes (Table 1) we combined four samples in September, two in October, and two for December through March. For sugar water consumption on the golf fairway (Table 2) we combined three samples for August 31 through September, four for October through December, and two for April through May. We combined the data from adjacent dates by using the mean values for each location or monitor over the combined time period. This procedure follows the repeated measures design and avoids inflating the sample size, with the *n* values the same as for single sampling dates. We then used analysis of variance on these means with multiple comparisons using the Tukey correction (Systat 1999). In all cases pretreatment comparisons of controls and treatments were not significant. Therefore, significant differences among treatments in subsequent dates imply significant changes from the pretreatment values. The means shown in the tables were back-transformed to their original units (Sokal & Rohlf 1995).

House treatments, sugar water consumption. We never found more than one ant species, and very few other insects, at a monitor. Occasionally, we found a pill bug or small beetle in the sugar water. In areas with heavy fire ant infestations, the ants could occasionally drain the contents of the monitors in 24 h. In most cases, when there was any consumption, the fire ants were still in the tube at time of collection. With a decrease in fire ant abundance after our treat-

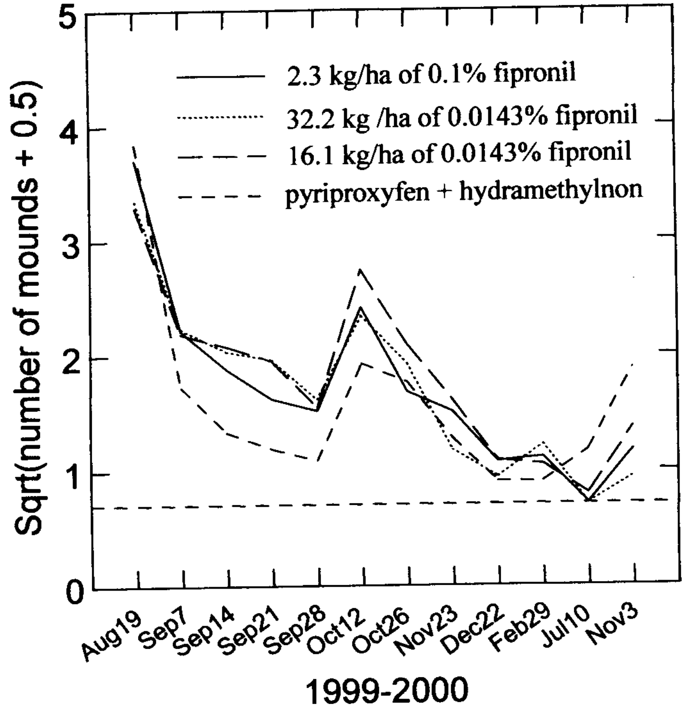

Fig. 3. Square root of number of mounds. The lower dashed line represents 0 mounds. The x-axis shows the sampling dates.

ments, we did find increasing numbers of other ant species at the monitors, particularly *Forelius* sp. (Hymenoptera: Formicidae) and another invasive species, *Tetramorium guineense* (Hymenoptera: Formicidae).

In most cases, we followed ant trails from the sugar water monitors back to small mounds within the plots, usually at distances of less than 3 m. We never observed ants at the monitors originating from outside of our plots. Visual search showed that most bait was gone after several hours of ant foraging. Furthermore, the corncob grit fire ant baits quickly lose attractiveness when wet. It is therefore unlikely that there was attractive bait was on the ground after the first evening's irrigation.

Table 1 shows the back-transformed means and multiple comparison statistics for the consumption of 25% sugar water over 24 h for each sampling period. One month after treatment, the bait treatments (Fig. 2, and Table 1, September) showed significantly greater reduction in ants than the combined fipronil treatments (reductions of 91% versus 81%, respectively), although fipronil was significantly different from the control. We attribute the reductions by the bait treatments to the hydramethylnon bait application because pyriproxyfen produces a slower effect. In October 1999, there were no significant differences in ant abundance among the treatments (although they were all statistically different from the control). The same was true for December 1999 through March 2000. Plots

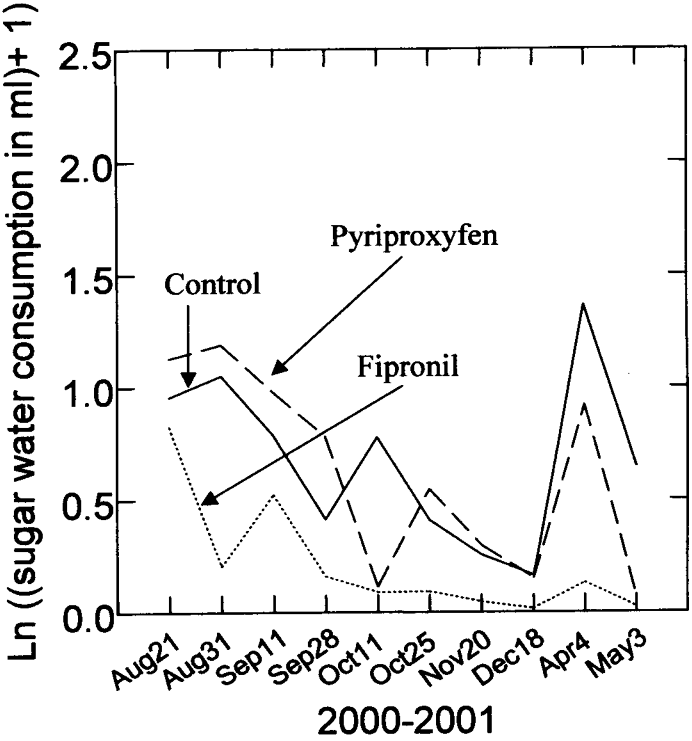

Fig. 4. Natural log of sugar water consumption on large fairway plot during 24 h. 0.1% granular fipronil applied at 2.3 kg/ha (12.5 lb/a); 276 g/ha (1.5 lb/a) of 0.5% pyriproxyfen. The x-axis shows the sampling dates.

that received the bait treatments showed some rebound in ant abundance on July 10, 2000 (10 months after treatment), with significantly higher consumption of sugar water than in the fipronil plots. At the end of the study in November 2000, reductions for the standard and fipronil treatments were 95% versus 99%, respectively. At this time, all the fipronil treatments had lower consumption than the bait treatments, although the differences were not significant using pairwise multiple comparisons. However, a statistical contrast of the three different fipronil treatments taken together as one group versus the bait treatment as another group was significant (F = 5.3; df = 1, 205; P = 0.03).

House treatments, mound counts. Mound counts are shown in Fig. 3. Because of the small sample sizes (n = 6 homes for each of the fipronil treatments and n = 8 homes for the bait treatments) and the similar results of the three fipronil treatments, we decided to do statistical contrasts of the three fipronil treatments as one group versus the standard bait treatment as another group. These comparisons were statistically significant one month after treatment (September), when the bait plots had significantly lower mound counts than the fipronil plots (reductions of 91% versus 73%; F = 9.2; df = 1, 22; P = 0.006).

Table 1. Consumption over 24 h of 25% sugar water (ml) at bait stations, Sunrise Country Club, Rancho Mirage, California, 1999 to 2000.

Treatment		Aug 19[a]	Sept	Oct	Dec–Mar	Jul 10	Nov 7
12.5 lbs/a of 0.1% fipronil	*(n)* $\bar{x}$	(36) 5.41 a	(40) 1.13 b	(38) 0.27 a	(40) 0.04 a	(37) 0.09 a	(39) 0.12 a
17.48 lbs/a of 0.0143% fipronil	*(n)* $\bar{x}$	(37) 5.97 a	(38) 0.89 b	(38) 0.49 a	(37) 0.050 a	(38) 0.05 a	(36) 0.03 a
87.4 lbs/a of 0.0143% fipronil	*(n)* $\bar{x}$	(36) 6.37 a	(38) 1.30 b	(37) 0.41 a	(38) 0.005 a	(37) 0.13 a	(35) 0.10 a
Pyriproxyfen + hydramethylnon	*(n)* $\bar{x}$	(66) 5.47 a	(68) 0.52 a	(68) 0.38 a	(68) 0.053 a	(68) 0.58 b	(64) 0.27 a
Control	*(n)* $\bar{x}$	(24) 5.37 a	(24) 5.81 c	(24) 3.53 b	(24) 1.02 b	(24) 0.66 b	(24) 1.29 b

Pairwise comparison of means for each date. In each column, means with the same letter are not significantly different (Tukey's multiple comparison test, $\alpha = 0.05$). For all the treatments, n is the number of sugar water bait stations, whether they are adjacent to homes or at the bases of trees; $\bar{x}$, the sample mean.

[a] Pretreatment values.

Table 2. Consumption over 24 h of 25% sugar water (ml) at monitors placed on the golf fairway, Las Palmas Country Club, Rancho Mirage, California, 2000 to 2001.

Treatment		Aug 21[a]	Aug 31–Sept	Oct–Dec	Apr–May
Fipronil	*(n)* $\bar{x}$	(12) 1.28 a	(13) 0.35 a	(13) 0.07 a	(13) 0.08 a
Pyriproxyfen	*(n)* $\bar{x}$	(10) 2.09 a	(11) 1.61 b	(11) 0.31 ab	(11) 0.57 a
Control	*(n)* $\bar{x}$	(36) 1.60 a	(36) 1.12 b	(36) 0.49 b	(36) 1.73 b

Pairwise comparison of means for each date. In each column, means with the same letter are not significantly different (Tukey's multiple comparison test, $\alpha = 0.05$). *n*, number of sugar water bait stations at the bases of trees; $\bar{x}$, the sample mean.

[a] Pretreatment values.

However, there were no significant differences during October ($F = 1.1$; df = 1, 22; $P = 0.3$) or during November through February ($F = 1.3$; df = 1, 22; $P = 0.26$). The increase in all mound counts in October 1999 (see Fig. 3) corresponded to the period when the summer grass was completely removed (scalped) before the planting of Bermuda grass for the winter. Evidently, very small mounds that were missed during earlier counts were now visible. In July 2000 (11 months after treatment) the fipronil-treated plots had fewer mounds ($F = 5.4$; df = 1, 22; $P = 0.03$). In November 2000 (15 months after treatment) the fipronil-treated plots again had fewer mounds numerically but not significantly so ($F = 3.9$; df = 1, 22; $P = 0.06$). At this time mound reductions for the bait and fipronil treatments were 79% versus 92%, respectively.

To correlate mound counts and sugar water consumption, we paired the average sugar water consumption measured at the monitors of each house with the number of mounds in its backyard, and pooled these data for all dates and houses. Decreases in ant abundance, as measured by sugar water consumption and mound counts, were significantly correlated during the experiment (Spearman's rank correlation, $r_s = 0.68$, $n = 286$, $P < 0.001$).

Fairway treatments. The fipronil treatment gave a more rapid reduction in sugar water consumption than the pyriproxyfen alone (see September 2000 sampling dates, Fig. 4). During the first month the former had reduced sugar water consumption by 68%, whereas the latter only reduced it by 33%. After nine months (May 2001) the respective reductions were similar at 98% and 97%. The fipronil treatments performed faster and had lower ant densities for most sampling periods (see Fig. 4 and Table 2). An overall repeated measures comparison of the posttreatment means for the fipronil and pyriproxyfen was significant ($F = 7.3$; df = 1, 49; $P = 0.01$).

Discussion

In our study, both mound counts and sugar water consumption gave treatment profiles consistent with the known properties of the tested pesticides. The bait treatments near the homes consisted of pyriproxyfen followed by hydramethylnon. The latter starts killing ants within a few days, while fipronil usually requires two to three weeks, and pyriproxyfen may require six weeks or more. In our data, after the house plots were treated with hydramethylnon, they showed both fewer mounds and less sugar water consumption in the month after the treatment than the fipronil-treated plots. However, on the fairway, the fipronil gave more rapid control than the pyriproxyfen bait applied alone (see "Fairway treatments" above). Although our plots around the homes were fairly narrow, if there had been cross-contamination of plots by the pesticides, we would not expect to see significant differences among treatments. The short field life of the baits because of irrigation and sunlight (Sullivan 2000, Bacey 2000) makes it unlikely that they could influence plots after application, and the granular fipronil, although longer lasting (Connelly 2001), only affects ants in the area where it is applied. Although ants could have moved during the course of the study to nearby plots, the fact that ant numbers were so reduced by all the treatments suggests that this kind of movement within the country club was unlikely. However, ants flying in from adjacent properties could have accounted for rebounds observed late in the study.

In general, near the homes, one application of fipronil worked as well as a single application of pyriproxyfen bait followed by a single application of hydramethylnon bait. The high fipronil treatment rate (32.2 kg/ha of the 0.0143% granular material) gave slightly quicker results in the measure using sugar water consumption, whereas the 2.3 kg/ha treatment with the 0.1% material gave faster results in reduction of mound counts. On the fairway, the fipronil outperformed the pyriproxyfen for most of the trial.

In Arkansas, there was a 99% reduction 6 weeks after treatment, with control still at 95% after 49 weeks using the 2.3 kg/ha of the 0.1% granular fipronil (Collins et al. 1999). Compared with these results, our California trials required more time to achieve maximal control, perhaps due to the differences in climate or irrigation. On these desert country clubs irrigation is on for 15 min four times per day.

Granular fipronil will be a useful addition to the list of pesticides available for RIFA in California. Although the product is more costly than the baits, fewer applications are necessary. It is ideal for golf courses where frequent irrigation, a necessity in the hot, arid conditions of the Coachella Valley, degrades other products, such as the fire ant baits used in this study. It is also easier to apply with spreaders already on hand, whereas the baits require a special spreader for the low rates at which they are spread.

Sugar water consumption, like other measures of ant abundance dependent on foraging activity, can be influenced by several factors. During cold and inclement weather when there is little foraging activity, it is not likely to be a very useful measure. Furthermore, the percentage of workers dedicated to foraging and the need for food will probably vary with the time of year and the amount of brood in the colony. In a field trial we are measuring an average response over a local population of colonies in a small contiguous area. It seems reasonable that foraging activity in this population will reflect relative ant abundance and thus indirectly allow for comparison of treatment effects among colonies. Food consumption by itself will not always give us an unbiased estimate of ant abundance, but it does allow us to compare treatment effects. More detailed field studies are needed to determine whether foraging activity or mound counts better reflect colony health over time.

In the hot, arid environment of the Coachella Valley, the fire ants readily consumed sugar water during most of the year, with the exception of the winter months. As a measure of ant abundance, sugar water consumption may not perform satisfactorily in climates different from California, and should be evaluated under local conditions.

Acknowledgments

Most of this work was funded by grants from the California Department of Food and Agriculture and the California Department of Pesticide Regulation. It was also funded in part by grants from Bayer Environmental Science, Montvale, New Jersey, and from Valent Professional Products, Walnut Creek, California. The authors thank Chris Olsen of Bayer Environmental Science for his assistance with this project, and the Sunrise and Las Palmas Country Club Homeowner's Associations for allowing us to use their property for the trials. We also thank Glenn Struckman and John Drouyor for assistance with the field project.

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