

FIPRONIL: AN ULTRA-LOW-DOSE BAIT TOXICANT FOR  
CONTROL OF RED IMPORTED FIRE ANTS (HYMENOPTERA:  
FORMICIDAE)

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ABSTRACT

Fipronil, a new broad spectrum pyrazole insecticide, was tested both in the laboratory and field as a bait toxicant for control of red imported fire ants, *Solenopsis invicta* Buren. Laboratory bioassays with worker ants showed that delayed toxicity occurred with baits ranging from 5 to 200 µg/ml active ingredient (AI). Tests with field-collected colonies in the laboratory confirmed the bioassay results with worker ants, and demonstrated that granular baits containing from 3.0 to 30 µg/mg (AI) eliminated colonies in 8 to 11 weeks after treatment. A field trial showed that a 15 µg/mg granular bait provided over 80% colony mortality at 6 and 12 weeks after broadcast application in non-grazed pastures. These results clearly demonstrate the potential of fipronil for use as a bait toxicant for control of red imported fire ants.

Key Words: *Solenopsis invicta*, imported fire ants, fipronil

RESUMEN

Fipronil, un insecticida pirazol nuevo de amplio espectro, fue probado en el laboratorio y en el campo en forma de cebo tóxico para el control de la hormiga importada de fuego, *Solenopsis invicta* Buren. Bioensayos de laboratorio utilizando hormigas obreras demostraron una toxicidad retrasada con cebos de 5 a 200 µg/ml de ingrediente activo (IA). Pruebas con colonias de hormigas obtenidas del campo confirmaron los resultados de los bioensayos con las hormigas obreras y demostraron que cebos granulares que contienen de 3.0 a 30 µg/mg (IA) eliminaron colonias entre 8 to 11 semanas después del tratamiento. Un experimento de campo demostró que un cebo granular de 15 µg/mg lograba más del 80% de mortalidad de la colonia entre 6 y 12 semanas después de haber sido distribuido el cebo al voleo en pasturas no en pastoreo. Estos resultados claramente demuestran el potencial de Fipronil para su uso como cebo tóxico para el control de la hormiga importada de fuego.

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Most insecticides are not suitable for use as bait toxicants for control of imported fire ants (*Solenopsis invicta* Buren or *Solenopsis richteri* Forel) due to the very rigid and exacting efficacy requirements. Stringer et al. (1964) noted that an effective bait toxicant must: (1) exhibit delayed kill over at least a ten-fold dosage range and preferably above a 100-fold dosage range; (2) be rapidly transferred from one ant to another via trophallaxis and kill the recipient; and (3) not be repellent to foraging ants.

Very few insecticides possess all three of these critical characteristics, and only those that do are acceptable for use in fire ant baits. At last count, more than 7,100 compounds have been tested as fire ant bait toxicants (Banks et al. 1992). However, only six of these toxicants have ever been commercialized, and two of those are no longer marketed. Bait toxicants that are registered and available for use today in-

clude: avermectin (ASCEND™, Whitmire Laboratories, St. Louis, MO), boric acid (BUSHWHACKER, Bushwhacker Associates, Galveston, TX) two fenoxycarb products (LOGIC® and AWARD™, Novartis Crop Protection, Inc., Greensboro, NC), three hydramethylnon formulations (AMDRO® and SIEGE®, American Cyanamid, Princeton, NJ, and MAXFORCE® Ant Killer Granular Bait, The Clorox Co., Oakland, CA).

Fipronil, 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(1R,S)-(trifluoroethyl)sulfinyl]-1H-pyrazol-3-carbonitrile, is a relatively new insecticide under development worldwide by Rhone-Poulenc AG Company (Research Triangle Park, NC). This compound is a member of the family of insecticides known as phenylpyrazoles (Moffat 1993). Fipronil has demonstrated potent insecticide and acaricide properties on a large number of pests including fleas, ticks, boll weevils, thrips, flies, and others (Colliot et al. 1992, Burris et al. 1994, Postal et al. 1995, Searle et al. 1995). Oral and dermal LD<sub>50</sub>s (rat) are 100 mg/kg and >2000 mg/kg, respectively (Colliot et al. 1992). We report here a series of trials in which fipronil was evaluated as a bait toxicant for control of red imported fire ants.

#### MATERIALS AND METHODS

##### Laboratory Test with Field Collected Red Imported Fire Ants Workers

Fipronil was tested as a bait toxicant for control of red imported fire ants in the laboratory using techniques and procedures described by Lofgren et al. (1967). A stock solution (0.17%) was prepared by adding technical fipronil (0.233 g) to Crisco® vegetable oil (138 g). The mixture was vigorously shaken and slowly heated to approximately 43°C. This process appeared to form a super-saturated solution (most of the active ingredient [AI] was dissolved, but some particles remained in suspension). Concentrations of 1600, 800, 400, 200, 100, 25, and 5 µg/ml were then prepared from the stock solution by serial dilutions with pure Crisco vegetable oil. All dilutions did appear to form true solutions.

Each concentration, plus a nontreated check, was tested against field-collected red imported fire ants workers in the laboratory. Four replicates of each treatment were tested. Each replicate consisted of 20 workers (mix of minors and majors), confined in test chambers consisting of plastic flower pots (5 × 5 cm) that had been furnished with a Labstone® (Bayer Corp. Dental Products, South Bend, IN) bottom, which wicked moisture from an underlying bed of damp peat moss. This arrangement provided a confined area with high humidity, which is necessary for survival of the ants. The vegetable oil baits were offered to the ants by soaking cotton balls (2 mm) with each concentration prior to placing in the test chambers. The cotton balls were removed from the test chambers after a 24 hr feeding period. Mortality was assessed at 0, 1, 3, 5, 7 and 14 days after treatment.

##### Laboratory Tests with Field Collected Whole Colonies

Results of the laboratory bioassay with red imported fire ant workers suggested that delayed toxicity occurred with 5-200 µg/ml baits. Several trials were then conducted by preparing a series of baits within this concentration range and testing them against whole colonies rather than small groups of isolated worker ants. Baits were prepared by serially diluting stock solutions of Crisco vegetable oil containing fipronil with pure vegetable oil to form different concentrations of oil and toxicant. These solutions were then used to impregnate inert carrier granules (defatted corn grits, Illinois Cereal Mills, Paris, IL). Formulated baits contained 30% vegetable oil/toxicant

and 70% inert carrier granules (w/w). Baits containing 3, 7.5, 15, and 30 µg/mg (AI) were prepared in this manner. Each concentration was then tested against field-collected fire ant colonies in the laboratory.

Colonies were confined in plastic pails (12-liter) and allowed to acclimate in the laboratory for 5 days before testing. Water was provided as needed, but no food was offered before testing. Queen status was not determined but each colony contained several thousand workers, immatures, and alates. Formulated bait (5 g) contained in a petri dish was placed on the surface of each of 3 colonies (replicates) for each bait concentration tested. The ants were allowed to feed *ad. lib.* on the baits for 24 hours at which time the petri dishes were removed and weighed, and the amount of bait removed from the petri dish by the ants was recorded. Colonies were maintained in the laboratory under ambient temperature, watered as needed, and provided food consisting of peanut butter and live mealworms. Quantitative data were not recorded, but colonies were observed weekly for 12 weeks. Behavioral changes such as feeding, colony maintenance, and mortality were noted and recorded.

#### Field Trial with Fipronil Bait

Based on results from the laboratory trials, a 15 µg/mg (AI) bait was formulated by Rhone Poulenc (Research Triangle Park, NC) for more intensive testing under field conditions. The test site was non-grazed pastures located in Harrison Co., MS. All baits were applied to test plots using a shop-built granular applicator mounted on a farm tractor. The experimental design was a completely randomized design (CRD) and there were 3 replicates per treatment. Treatments included the fipronil bait which was applied at rates of 1.7 and 3.4 kg formulated bait/ha (25.5 and 51.0 mg AI/ha, respectively), a hydramethylnon standard (Amdro®, 0.73% AI bait, American Cyanamid, Princeton, NJ) applied at 1.7 kg formulated bait/ha, and a nontreated check. All test plots were 0.4 ha in size except for the fipronil plots which were 0.2 ha.

Before treatment, circular subplots with a radius of 17.9 m (0.1 ha) were established in the center of each test plot. Imported fire ant population estimates were made in each circular subplot before and 6, 12, 18, and 24 wks after pesticide application, using the population indexing system described by Harlan et al. (1981) and modified by Lofgren and Williams (1982). As shown in Table 1, this system is based on the estimated population of worker ants and the presence or absence of worker brood (larvae and pupae). Absence of worker brood suggests that a colony does not contain a normally functioning queen. A newly-formed colony with worker brood present and less than 100 workers is numerically weighted as a "5" (colony class 6). Colonies of this rating are not easily visible in the field due to their very small mound size and, thus, rarely are detected. A large mature colony with worker brood and more than 50,000 workers is assigned a weighting factor of "25" (colony class 10). The population index for a particular site is calculated as follows:

$$\text{Population Index (PI)} = \sum_{K=1}^{25} K(N_K)$$

where  $N_K$  = the number of imported fire ant colonies in a given plot with a weighting factor of  $K$  where ( $25 \geq K \geq 1$ ). The number of active imported fire ant mounds ( $\geq 20$  workers) and population indices were calculated for each subplot. Nontreated check plots were not treated in any manner, but were evaluated using the population estimation method previously described. These data were used to determine: 1) colony mortality, which is the percentage decrease in the pretreatment number of active mounds at each assessment interval; and 2) the percentage change in the pretreat-

TABLE 1. COLONY CLASSIFICATION SYSTEM USED TO EVALUATE THE EFFECTS OF INSECTICIDES ON IMPORTED FIRE ANT POPULATIONS.

Number of worker ants	Worker brood absent		Worker brood present	
	Colony class	Weighting factor	Colony class	Weighting factor
<100	1	1	6	5
100-1,000	2	2	7	10
1,000-10,000	3	3	8	15
10,000-50,000	4	4	9	20
>50,000	5	5	10	25

ment population indices at each assessment interval. Percentages were arc sine transformed and substitutions for values of 0% and 100% were made as stated by Gomez & Gomez (1984) before transformation. Transformed data for each treatment were separated using ANOVA and a Tukey's test (SPSS Inc. 1992).

#### RESULTS AND DISCUSSION

##### Worker Bioassay in the Laboratory

The 5 µg/ml rate did exhibit delayed toxicity, providing <25% mortality at 3 days after treatment (Fig. 1), and 97.5% mortality by 14 days after treatment. Higher concentrations (≥400 µg/ml) rendered much faster kill, approaching 100% mortality within 3 days, which is not desirable with bait toxicants as explained by Stringer et al. (1964). The intermediate concentrations (25-200 µg/ml) did not result in the rapid kill seen at higher rates, but did kill much faster than the 5 µg/ml rate.

##### Whole Colony Trials in the Laboratory

Granular baits containing 3, 7.5, 15.0, and 30 µg/mg (AI) were readily fed upon by test colonies in the laboratory. Some repellency was noted at 30 µg/mg because not all bait was removed by foraging workers. Delayed toxicity at each dose tested was evident by the progressive decline in number of active workers and large increase in number of cadavers. Maintenance and repair of the treated nests ceased. As an example, the routine addition of water to the nest created craters in the nest surface which were not sealed off or repaired. Check colonies performed this task within 1 to 2 hours after the craters were formed. All treated colonies died within 8 to 11 weeks after consuming the fipronil baits.

##### Field Trial with Fipronil Bait

The 15 µg/mg (AI) fipronil bait applied at either 1.7 or 3.4 kg formulated bait/ha (25.5 or 51.0 mg AI/ha, respectively) provided >96% reduction in pretreatment population indices at 6 and 12 weeks after application (Table 2). Colony mortality with both rates of application was >80% (Table 3). Before the 18 week evaluation, part of the property was lightly disked, and as a result, one hydramethylnon-treated plot and

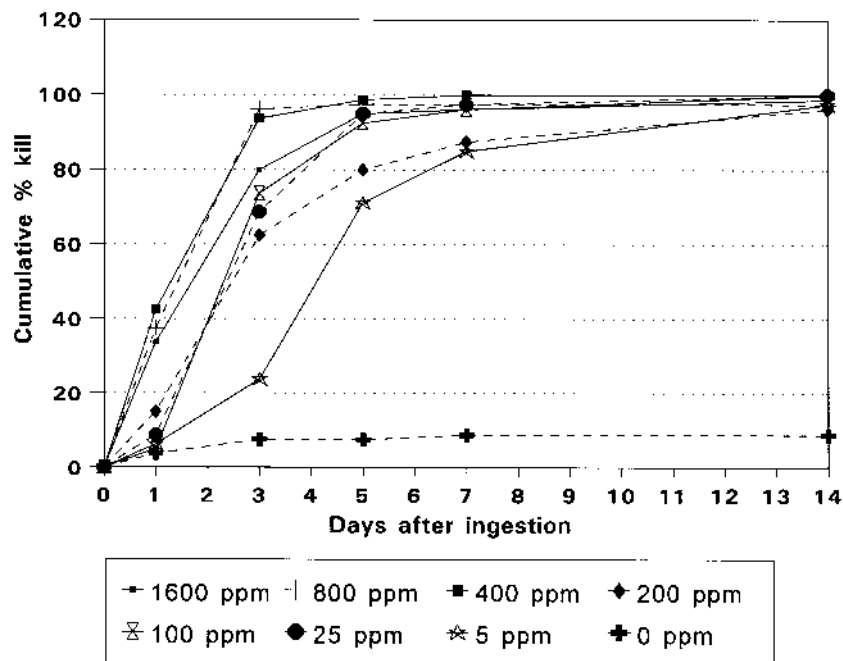


Fig. 1. Cumulative mortality to red imported fire ant workers after feeding on various concentrations of fipronil dissolved in Crisco® vegetable oil.

one nontreated check plot were lost. Therefore, treatment means for the hydramethylnon and check evaluations at 18 weeks were based on 2 replicates, rather than 3. At 18 weeks after treatment, the 1.7 kg fipronil rate provided 94.2% reduction in pretreatment population indices and 87.5% reduction in pretreatment colony numbers. The 3.4 kg rate of fipronil and the hydramethylnon standard were both reinfested with healthy colonies 18 weeks after treatment. Presence of small, incipient colonies indicated that reinfestation of all test plots had occurred. Neither fipronil rate of application was statistically different from the hydramethylnon standard at any time during this trial (Tables 2 and 3).

We have previously reported the importance of using relatively large test plots (at least 0.4 ha in size) when evaluating toxicants for control of red imported fire ants. Plots this large provide a treated buffer area of 14.2 m, which minimizes colony relocation into the test plots from adjacent nontreated areas (Collins & Callcott 1995). However, treated buffers as large as 75 m do not necessarily prevent movement from outside the treated area because Callcott & Collins (1992) noted the appearance of large, mature colonies in treated test plots with a 75 m treated buffer approximately 16 weeks after treatment. Plot size for the fipronil plots was 0.2 ha due to limited availability of the experimental bait, and therefore, these plots had treated buffers of only 4.8 m. Although overall plot size was reduced for the fipronil plots, the evaluation areas (0.1 ha) were consistent in all plots in this trial. The few remaining colonies in fipronil plots did not contain worker brood 6 and 12 weeks after treatment, indicating abnormal colonies and the apparent absence of a functioning queen. However, all of

TABLE 2. EFFECTIVENESS OF FIPRONIL BAIT AGAINST FIELD POPULATIONS OF RED IMPORTED FIRE ANTS.

Treatment	Rate of applic. (kg/ha)	Mean pop. index $\pm$ SEM - pretreat*	% change in mean population indices at indicated weeks after treatment**			
			(6)	(12)	(18)	(24)
Fipronil 0.0015%	1.7	205.0 $\pm$ 60.6	-96.6a	-96.9a	-94.2a	-67.1a
Fipronil 0.0015%	3.4	315.0 $\pm$ 18.0	-97.3a	-96.2a	-82.4a	-62.8a
Hydramethylnon 0.73%	1.7	353.3 $\pm$ 153.7	-90.1a	-88.3a	-82.4a <sup>†</sup>	-76.0a
Nontreated Ck	—	156.7 $\pm$ 45.1	-36.3b	-28.2b	12.0b <sup>†</sup>	54.4b

\*Mean based on 3 replicates; see text for definition of population index method.

\*\*Means within a column followed by the same letter are not significantly different (Tukey's test,  $P \leq 0.05$ ; on arc sine transformed data).

<sup>†</sup>Before the 18 week count, one hydramethylnon replicate and one nontreated check replicate were lost due to pasture improvements, therefore only 2 replicates were included in these means.

TABLE 3. EFFECTIVENESS OF FIPRONIL BAIT AGAINST FIELD POPULATIONS OF RED IMPORTED FIRE ANTS.

Treatment	Rate of applic. (kg/ha)	Mean no. colonies present $\pm$ SEM - pretreat*	% decrease in mean no. of colonies at indicated weeks after treatment**			
			(6)	(12)	(18)	(24)
Fipronil 0.0015%	1.7	15.7 $\pm$ 5.1	81.9ab	87.5a	87.5a	61.6a
Fipronil 0.0015%	3.4	21.7 $\pm$ 2.1	84.1a	84.6a	68.6a	56.9a
Hydramethylnon 0.73%	1.7	29.3 $\pm$ 16.2	77.4ab	79.3a	79.6a <sup>†</sup>	76.1a
Nontreated Ck	—	12.7 $\pm$ 3.2	47.5b	36.3b	3.6b <sup>†</sup>	0.0b

\*Mean based on 3 replicates; see text for definition of population index method.

\*\*Means within a column followed by the same letter are not significantly different (Tukey's test,  $P \leq 0.05$ ; on arc sine transformed data).

<sup>†</sup>Prior to the 18 week count, one hydramethylnon replicate and one check replicate were lost due to pasture improvements, therefore only 2 replicates were included in these means.

the plots treated at the 3.4 kg rate contained 1-4 large, normal colonies (colony class 8 according to the population index scale of Lofgren & Williams 1982) 18 weeks after treatment. While it is possible for these colonies to have developed from newly mated queens entering the treated area just after treatment, it is more likely that they migrated into test plots from the adjacent nontreated area (Markin et al. 1973, Callcott & Collins 1992). Adoption of newly mated queens by surviving broodless colonies could also account for the sudden appearance of category 8 nests within the test plots.

#### DISCUSSION

Laboratory tests with the new pyrazole insecticide fipronil indicated good potential for this compound as a bait toxicant against the red imported fire ant at rates of 3-30 µg/mg. The field trial showed that fipronil applied as a 15 µg/mg bait (0.0015% [AI]) at 1.7 or 3.4 kg/ha, controlled red imported fire ants as well as the hydramethylnon standard which was applied as a 0.73% (AI) bait at 1.7 kg/acre. These results indicate that fipronil met the criteria for effective fire ant bait toxicants that were listed by Stringer et al. (1964). Specifically, fipronil was effective over more than a 10-fold dosage range, was transferred throughout colonies via trophallaxis, and was not repellent to foraging workers.

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