Effects of Boric Acid, Fipronil, Hydramethylnon, and Diflubenzuron Baits on Colonies of Ghost Ants (Hymenoptera: Formicidae)

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ABSTRACT Laboratory colonies of the ghost ant, *Tapinoma melanocephalum* (F.) were administered sugar solution (10%) baits containing the insecticides boric acid, fipronil (REGENT), hydramethylnon (SIEGE), or diflubenzuron (DIMILN). Colonies were exposed to the baits for 21 d, and development of workers, queens, and brood (larvae and pupae) was observed for 4 wk. Fipronil (0.05%) caused 100% mortality in all colonies the first week. With boric acid (0.5%), 100% mortality of workers, queens, and brood was reached at the end of the third week. With hydramethylnon (2%), 83% of the colonies disappeared at the end of the fourth week, but some queens were still alive 9 wk after the trial started. Diflubenzuron (1%) behaved similarly to the control, although in some colonies, the brood production increased, whereas in other colonies, the queens disappeared. In the control colonies, workers, queens, and brood were always observed even up to 9 wk.

KEY WORDS *Tapinoma melanocephalum*, toxic baits, insecticides, ants

AMONG THE EIGHT MOST important species of urban ants in the Valley Province (Colombia), the ghost ant, *Tapinoma melanocephalum* (F.), and the crazy ant, *Paratrechina longicornis* (Latreille), are the most important species. The ghost ant was found in high frequencies in hospitals (51.3%) and in residential areas (28.5%) in the seven major cities in this province. In residential zones, the ghost ant was found primarily in association with kitchen, dining room, and bathroom areas; in hospitals and clinics, the frequency was greater in the emergency rooms, kitchens, and pediatric sections (Lozano et al. 1999, Lozano and Chacón de Ulloa 2001). A study directed at identifying the potential of several species of urban ants as vectors of pathogenic agents in hospitals of the Cauca Valley indicated that ghost ants were capable of transporting seven types of bacteria, such as *Enterobacter cloacae* (Jordan) and *Staphylococcus* sp. (Olaya and Chacón 2001).

Few studies have been conducted on the chemical control of the ghost ant, despite that it is a widely distributed urban species that is found primarily in tropical zones but also in temperate zones of both the new and old worlds (Robinson 1996, Bustos and Cherix 1998). Klotz et al. (1996) tested, for the first time, the effect of liquid baits containing boric acid (1%) on small laboratory colonies of ghost ants and found a 90% reduction of worker and brood populations. They considered boric acid promising for controlling the species and recommended conducting further studies to test these toxic baits on large field and laboratory colonies. Klotz et al. (1996) also observed that a solid form of hydramethylnon (0.9%) had no lethal effects on ghost ant colonies.

This study evaluated the effect of four insecticides added to liquid baits on laboratory colonies of ghost ants: boric acid, fipronil (REGENT), hydramethylnon (SIEGE), and diflubenzuron (DIMILN).

Materials and Methods

Insects. Ants were collected from nests located around garden plants, and a large number of workers, some queens, and alates were attracted to protein baits and honey, located in kitchen areas in homes in the urban area of Cali, Colombia. Ants were taken to the Entomology Laboratory at the Universidad del Valle (26°C, 80% RH); 30 colonies were formed, using a methodology developed by Jaramillo and Chacón (2003). Each colony was conditioned in a plastic box ($17 \times 17 \times 8$ cm), the walls of which were covered with Teflon to prevent workers from escaping. Inside the box there were areas for foraging and nesting. The nest was made from a plastic petri dish (6 cm diameter), the bottom of which was covered with blue cardboard divided into 1-cm squares (nest area: 28 cm^2) to facilitate observations. To create darkness, the nest was covered with an inverted plastic cup lined with black contact paper with small holes around the edge to permit the entry and exit of the workers. In the foraging area (250 cm^2), water was made available ad libitum in eppendorf tubes stopped with cotton plugs. Pieces of food rich in proteins and carbohydrates (chicken, beef, cooked egg yolks, and honey) were

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Table 1. Loss of weight in baits with and without exposure to the ant colonies

Bait contents (sugar solution + insecticide)	Loss of weight in baits (mean ± SD/g)		Mann–Whitney test
	Exposure to ants (n = 6)	No exposure to ants (n = 4)	$U_{0.05(2),\ 4,\ 6} = 22$
Diflubenzuron (1%)	0.82 ± 0.28	0.20 ± 0.0	U = 30, $P < 0.002$
Boric acid (0.5%)	0.68 ± 0.39	0.13 ± 0.05	U = 24, $P < 0.01$
Control	0.67 ± 0.31	0.30 ± 0.22	U = 22, $P < 0.05$
Fipronil (0.05%)	0.52 ± 0.04	0.40 ± 0.37	U = 18, NS

NS, not significant.

supplied every other day. Colonies were maintained for ≈2 mo, after which they were used to test the chemical products.

Insecticide Treatments. Four insecticides were tested: fipronil (Regent 200 Concentrate Suspension 20%; Aventis CropScience, Bogotá, Colombia); hydramethylnon (Siege gel insecticide 2%, American Cyanamid, Princeton, NJ); diflubenzuron (Dimilin Wettable powder 25%, Proficol; El Carmen, Uniroyal Chemical, Bogotá, Colombia); and crystalline boric acid (99% [AI], IMC, Chemical Industrial, Taipei, Taiwan).

To prepare the baits, the products were diluted in a sugar-water solution (10%), except for hydramethylnon, which was used directly in its commercial presentation (gel). The concentration of the products in the sugar solution was as follows: fipronil (0.05%), diflubenzuron (1%), and boric acid (0.5%). The liquid baits were placed in eppendorf tubes stopped with a cotton plug, whereas eight drops of the gel containing hydramethylnon were placed in each colony on a petri dish that was removed after the third week.

Thirty colonies consisting of queens, workers, and brood (larvae and pupae) were formed and subdivided into three groups according to the density of workers found in the nest. Each treatment had colonies from the three groups. Six colonies were tested for each of the four insecticides; the six control colonies received only a sugar solution (10%). The colonies were starved for 24 h before they had access to the baits. The baits were administrated continuously for 3 wk and were changed every week. During the 3-wk administration period, the colonies were also offered other kinds of food. After the third week, the colonies returned to the normal diet (honey, chicken, and others).

To determine whether the ants had consumed the liquid baits during the first 2 wk, the weight loss of the baits was calculated and compared with that of baits left under ambient conditions in the laboratory. This methodology is a modification of that published by Klotz et al. (1996). From the onset of the trial, the workers, queens, and brood that were alive in the 30 colonies were counted weekly for 4 wk starting the first day of baiting, and photographs were taken with a digital camera (Sony DSC-S70, Sony Corporation, Tokyo, Japan). The photographs corresponded to the nesting and foraging area, and the dead individuals that were in the refuse area were not included in the countings. The resulting images were analyzed with specialized software (Q-Win ver. 2.0, Leica Imaging Systems, Cambridge, England), and the counts of brood, workers, and queens were done using the multiple-measures tool. Qualitative observations to detect a possible colony recuperation were made from the fourth to the ninth weeks.

Statistical Analysis. Weight losses of the baits were compared with the Mann–Whitney test (Zar 1996). To determine differences in the survival of workers, queens, and brood between bait treatments, a one-way analysis of variance (ANOVA) was done, and the means were separated by the Tukey honestly significant difference (HSD) test on transformed data ($X' = \sqrt{X + 1}$) (Zar 1996). To determine differences in the survival of workers, queens, and brood for each treatment over the 4-wk period, an ANOVA by ranks using Kruskall–Wallis statistic (H), with χ^2 approximation, followed by multiple comparisons of means ranks, was performed (Zar 1996). Statistix for Windows (1998) was used for all analyses. To calculate the LT_{50} and LT_{90} of workers, the data were corrected with the formula of Abbott (1925) and analyzed by Probit analysis (Raymond 1985).

Results

Consumption of Toxic Baits. Significant differences (Mann–Whitney U test) between the average weight of the baits placed in the ant colonies and those kept at ambient temperature in the laboratory were found for diflubenzuron and boric acid as well for the control (Table 1). No differences were found among the baits containing fipronil. With respect to the hydramethylnon gel, qualitative observations revealed that the ants did consume it.

Insecticidal Activity. The ANOVA analysis showed significant differences in the survival of workers, queens, and brood among treatments (Table 2). The mean number of workers alive was significantly lower in colonies treated with fipronil, boric acid, and hydramethylnon, but was similar in control and diflubenzuron colonies (Tukey HSD test). The mean numbers of live queens and brood were both significantly lower in colonies treated with boric acid and fipronil followed by hydramethylnon and diflubenzuron, and these two products did not show significant differences with the control.

A more detailed analysis of the age-class distribution of the populations in the colonies showed that insecticidal action differed over the 4-wk period, depending on the different development stages of the ants (workers, queens, and brood) (Figs. 1, 3, and 4). Figure 1 illustrates average survival of workers. The

Table 2. Comparison of mean ± SEM of workers, queens, and brood alive for *T. melanocephalum* colonies during exposure to boric acid, diflubenzuron, fipronil, and hydramethylnon baits

Bait treatment	*n*	No. workers alive[a]	No. queens alive[a]	No. brood alive[a]
Boric acid	30	111.5 ± 44.1b	0.53 ± 0.14b,c	6.7 ± 2.7b
Diflubenzuron	30	219.2 ± 29.0a	1.03 ± 0.18a,b	31.4 ± 6.3a
Fipronil	30	64.8 ± 32.2b	0.30 ± 0.12c	8.3 ± 4.5b
Hydramethylnon	30	61.8 ± 14.8b	1.20 ± 0.24a,b	16.8 ± 4.2a,b
Control	30	284.2 ± 50.3a	1.37 ± 0.26a	27.4 ± 5.6a
P		0.0001	0.001	0.0001
df; *F*		4, 145; 15.36	4,145; 5.42	4,145; 7.75

[a] Means within a column followed by the same letter are not significantly different ($P > 0.05$, Tukey HSD test; Zar 1996).

control showed a decrease in the survival rate, but all the colonies had workers at the end of the fourth week, and no significant differences were found in the survival rate from 1 wk to another (Kruskall–Wallis $H = 7.37$; $P > 0.05$; approximated by χ^2, critical value χ^2 0.05, 4 = 9.488). For diflubenzuron, a gradual decrease in the survival rate was also observed, but toward the end of the fourth week, five of the six colonies had workers, and highly significant differences were found in the survival rate from 1 wk to another ($H = 13.84$, df = 4, $P < 0.01$). The boric acid and the hydramethylnon behaved similarly: the survival rate fell significantly in both treatments ($H = 21.87$, df = 4, $P \ll 0.001$ and $H = 24.37$, df = 4, $P \ll 0.001$, respectively) during the first 2 wk, but in the case of the boric acid, only one colony with workers remained at the end of the second week. This colony died in the third week (Fig. 2). With hydramethylnon, there were three colonies with workers at the end of the second week; one of them still had workers at the end of the observations. With fipronil, significant differences were also found ($H = 28.54$, df = 4, $P \ll 0.001$), and this was the insecticide that acted most rapidly; all colonies lost their workers in the first week of the treatment.

Table 3 gives the average values of the probit analysis for workers. This analysis was not done for fipronil because, given its rapid action, it was not possible to obtain a sufficient number of points for analysis.

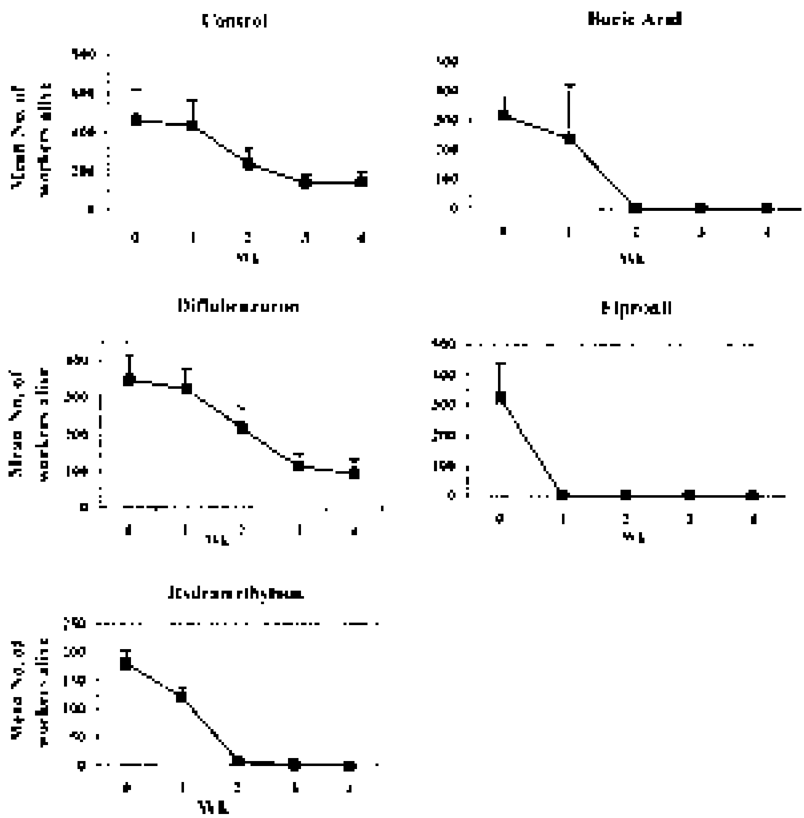

Fig. 1. Populations of workers in colonies of *T. melanocephalum* treated with four insecticides and in control colonies. Values refer to the average of the six colonies for each treatment plus the SEM.

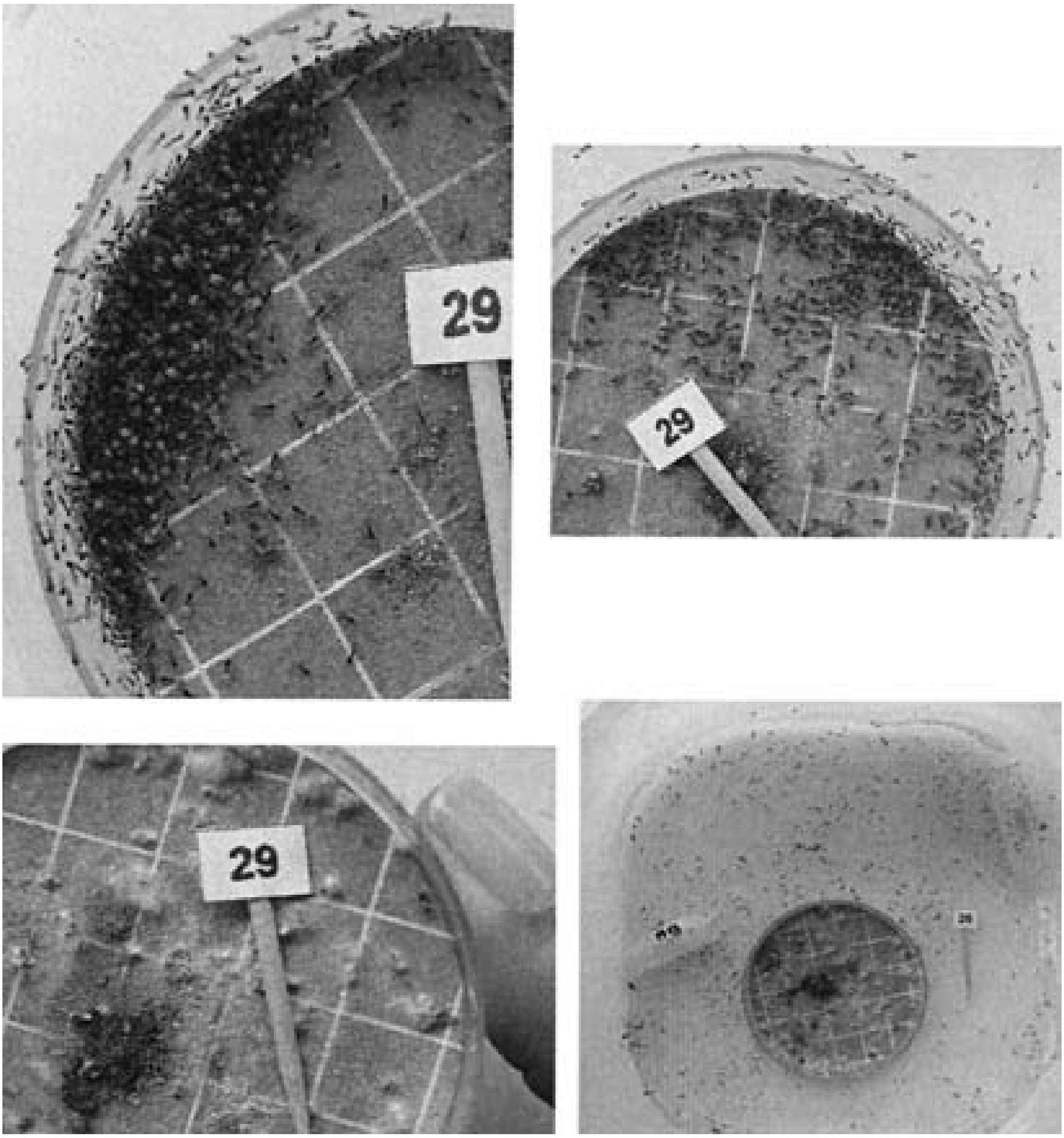

Fig. 2. Colonies of *T. melanocephalum* treated with boric acid. The pictures correspond to 3 wk of observations.

No significant differences were found between the LT_{50} of workers for hydramethylnon and boric acid, but these two did show differences when they were compared with diflubenzuron (18.4 d). The same was true for the LT_{90}, where diflubenzuron took more time (44.58 d) to eliminate 90% of the population. Hydramethylnon and boric acid behaved similarly for both the LT_{50} and the LT_{90}. In general, diflubenzuron acted much more slowly than hydramethylnon and boric acid.

Figure 3 illustrates the average survival rate of the queens for each treatment. The control showed a

Table 3. LT_{50} **and** LT_{90} **of workers for hydramethylnon, diflubenzuron, and boric acid**

Bait	LT_{50} (95% CL; d)	LT_{90} (C 95% CL; d)	Slope ± SE	No. ants (average)	χ^2	*P*
Hydramethylnon	8.23 (7.7–8.7)	13.57 (12.61–14.85)	5.8 ± 0.44	179.5	3.55	0.83
Diflubenzuron	18.4 (17.5–19.3)	44.58 (40.1–50.63)	3.33 ± 0.19	348.17	0.55	0.54
Boric acid	8.4 (8.1–8.7)	11.74 (11.06–12.64)	8.84 ± 0.61	315	3.65	0.15

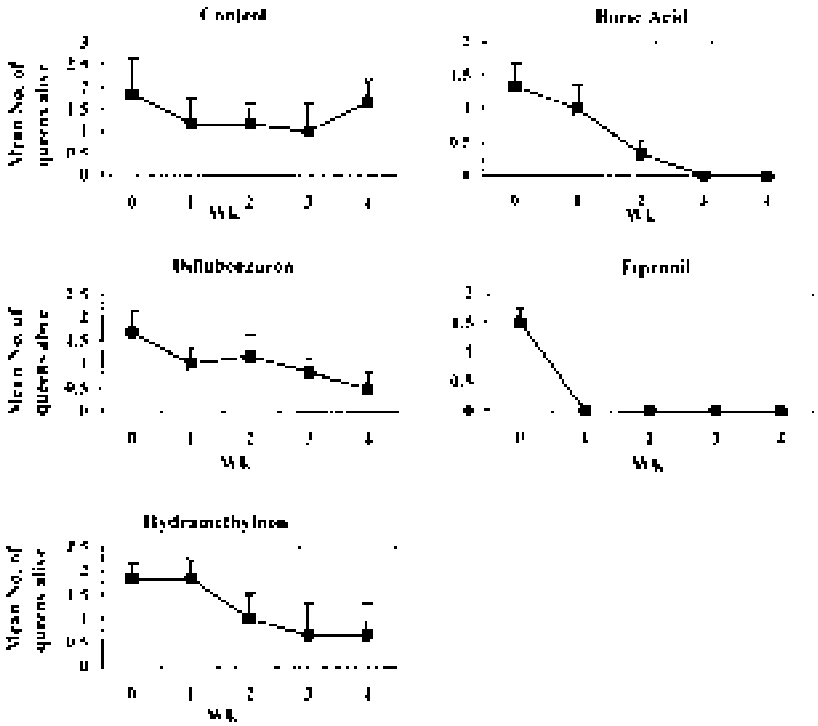

Fig. 3. Populations of queens in colonies of *T. melanocephalum* treated with four insecticides and in control colonies. Values refer to the average of the six colonies for each treatment plus the SEM.

decrease in the number of queens for the first week, after which it was constant; there were even new queens by the end of the fourth week. In the case of diflubenzuron, all the colonies had queens in the first 2 wk, but by the end of the fourth week, there were four colonies with queens. Hydramethylnon showed greater variation in the number of queens from 1 wk to another, but no significant differences were found ($H = 8.85$, df $= 4$, $P > 0.05$). The queens began to disappear by the second week, when only three colonies had queens. Toward the end of the fourth week, only one colony had queens, which survived until the end of the ninth week. With the boric acid, the decline in the number of queens was progressive, and significant differences were found in the survival rates from 1 wk to another ($H = 15.06$, df $= 4$, $P < 0.005$); at the end of the second week, only one-half of the colonies still had queens, and these disappeared totally by the third week. The fipronil acted rapidly on the queens; all died within the first week. Significant differences were also found ($H = 28.64$, df $= 4$, $P \ll 0.001$).

Figure 4 shows the brood (larvae and pupae) populations in each treatment during the 4-wk monitoring period. In the control, larvae and pupae in the six colonies showed great variations, although brood was always present within all colonies throughout the study. Therefore, no significant differences were found ($H = 1.11$, df $= 4$, $P > 0.05$). In the treatment with diflubenzuron, brood from two colonies disappeared after the second week but tended to increase in the other four colonies. In fact, after 9 wk of observations, three colonies still had brood. In the third week it was observed that some eggs and larvae were light brown in color (the same color as the bait), but their size and general appearance seemed normal. In fact, the larvae were being fed normally by the workers. Significant differences were found with the boric acid and the hydramethylnon ($H = 19.26$, df $= 4$, $P < 0.001$ and $H = 13.55$, df $= 4$, $P < 0.01$, respectively). There was a marked decrease in the broods for the first 2 wk, but the decrease was more rapid with the boric acid because at the end of the second week there was only one colony with a brood, which disappeared during the third week. In the case of hydramethylnon, there were three colonies with brood at the end of the second week, and one colony had a brood until the fourth week. In the case of fipronil, significant differences were also found ($H = 22.95$, df $= 4$, $P \ll 0.001$); all colonies lost their brood in the first week.

Discussion

Toxic baits prepared with insecticides added to a sugar solution were attractive to the ghost ants and were appropriate for implementing trials for controlling the species, similar to the Argentine ant, another species of the subfamily Dolichoderinae (Hooper-Bui and Rust 2000). The significant differences observed in the weight loss between baits placed in the ant colonies and those left under ambient conditions indicate that workers consumed the sweetened baits containing insecticides. No differences were found

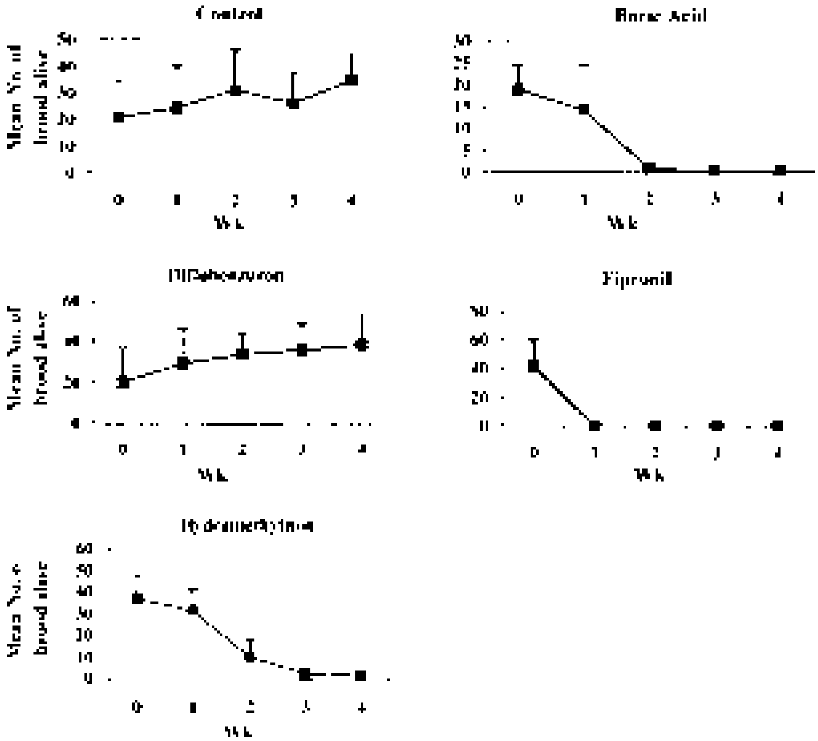

Fig. 4. Populations of brood (larvae and pupae) in colonies of *T. melanocephalum* treated with four insecticides and in control colonies. Values refer to the average of the six colonies for each treatment plus the SEM.

among baits with fipronil, which is because the colonies died very quickly (week 1) and therefore the workers could not finish consuming the bait.

Two insecticides were promising in the control of ghost ants: fipronil (0.05%) and boric acid (0.5%), although the second acted more slowly. Of the colonies treated with fipronil, 100% lost all their workers, queens, and brood in the first week of the trial. On the sixth day after having placed the bait, the six colonies had all died. Such rapid action observed with fipronil was probably caused by the high rate used, given that in studies conducted with the Argentine ant (*Linepithema humile* [Mayr]), lower rates were also effective (Costa and Rust 1999). They observed that in 1 wk, 90% mortality was reached in workers that were foraging on plants treated with fipronil (5 and 10 ppm AI); mortality in queens was 100% when exposed to 5 ppm fipronil and 89% with 10 ppm fipronil. Hooper-Bui and Rust (2000) observed that in baits administered for only 24 h to Argentine ants, the fipronil (10^{-5}%) caused 100% mortality of workers. At lower rates (10^{-6}%) with continuous exposure to the toxic baits over a 2-wk period, the same result was obtained. With the queens, fipronil (10^{-5} and 10^{-4}%) caused 100% mortality in 24 h; in continuous exposure to the toxic baits, rates of 10^{-5}% caused 100% mortality in both the queens and the workers.

Five of the six colonies treated with boric acid (0.5%) for 21 d lost 100% of their workers and brood by the second week. In the sixth colony, this occurred in the third week. With respect to the queens, one-half the colonies had no queen at the end of the second week; by the third week, 100% mortality was also reached. The time in which 100% disappearance of the colonies occurs depends on the rate of insecticide application and on the time of exposure. Klotz et al. (1996) observed that small colonies of *T. melanocephalum* exposed for only 3 d to sugar solution baits, but with double the boric acid (1%), reduced their populations of workers by 97% in the first week, the brood by 96% in the third week, and the queens were eliminated in week 12. The same authors note that continuous exposure to the same rate of boric acid was slightly more rapid in eliminating workers, queens, and brood.

Studies carried out on the control of the Argentine ant in the field showed a continuous reduction of the population (up to 80%) when boric acid (0.5%) in a 25% solution of sugar was used for 10 wk (Klotz et al. 1998). Using the same rate for only 2 wk of exposure, Hooper-Bui and Rust (2000) recorded 100% mortality of worker and queen Argentine ants. They also reported that exposure to boric acid at lower (0.25%) or higher (0.75%) rates for 24 h was not effective. Very high concentrations of boric acid should not be used, given that high rates increase the rapid death of workers, thereby reducing trophallaxis (Klotz et al. 1997), preventing the toxic substance from reaching the colony (i.e., queens and brood). These factors minimize the potential effectiveness of this toxic bait.

Hydramethylnon (2.0%) caused much lower toxicity, and its action was slower. The total loss of workers, queens, and brood occurred in only 50% of the colonies at the end of the second week and did not reach 100% at the end of the trial, given that one of the colonies still had workers, queens, and brood. In addition, some queens were still alive 9 wk after the trial began. In one of the colonies, five queens, one alate, and one worker managed to survive; however, despite the fact that they seemed to be in good condition, when a test for oviposition was conducted, none of these queens had oviposited (24 h later). However, our results showed greater activity of hydramethylnon than that observed by Klotz et al. (1996). They tested a solid formulation of 0.9% hydramethylnon (in silkworm pupae) and found that not one ghost ant colony had been eliminated after 10 wk of treatment. The differences between our observations and those of Klotz et al. (1996) may be because of the fact that we used higher rates of the insecticide (2%). At the same time, Zhakharov and Thompson (1998) observed that the sensitivity to the continued use of hydramethylnon in native ants from the subfamily Dolichoderinae, among which is found the genus *Tapinoma,* was relatively low. In contrast, Hooper-Bui and Rust (2000), who worked with the Argentine ant, found that hydramethylnon (0.1%) caused 100% mortality in workers at the end of 24 h; at even much lower rates (0.001–0.05% and with continuous exposures for 2 wk), the mortality of workers was 60–85% after 2 wk. These data contrast with the aforementioned studies and with the results obtained in our trial with the ghost ant, suggesting that more precise experiments should be conducted to determine appropriate rates and times of exposure for this species.

Diflubenzuron is not recommended for controlling the ghost ant, given that the total loss of workers was observed in only 17% of the colonies and the mortality of queens in 33%. In addition, brood continued developing in 50% of the colonies 9 wk after initiating the treatment. Our results are in agreement with the observations made by Catangui et al. (1996) of the congeneric species, *T. sessile* (Say), whose populations were not significantly reduced when aerial applications of diflubenzuron were made to control grasshoppers in fields of mixed-grass.

The use of liquid sugar baits, to which insecticides such as fipronil and boric acid are added, can be considered promising for control of the ghost ant. It is necessary to carry out studies directed at the use of lower rates of fipronil and conduct experiments with both products under "natural" conditions; that is, in urban areas where this ant is an important pest.

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