Foraging Populations of Tube Building Termites, *Gnathamitermes perplexus* (Banks), Associated With Termiticide Experiments in Southern Arizona (Isoptera: Termitidae)

by

Javier G. Miguelena[1] and Paul B. Baker[1,2]

ABSTRACT

In the southwestern desert region of Arizona, a common non-structure invading species is the tube building termite *Gnathamitermes perplexus*. It is a valuable species from an ecological point of view due to its role in the decomposition of dead wood in desert environments and its capacity to enrich and aerate vast quantities of soil. Since it has no economic importance, very little is known of the effects of termite control measures on it. However, *G. perplexus* is likely exposed to termiticides used to manage more damaging termite species with which it co-occurs. Since most common termiticides have relatively generalized modes of action, we hypothesized that *G. perplexus* populations would decrease significantly as a result of termiticide application. The results reported here are part of a larger study in which we were primarily interested in evaluating foraging termite populations of *Heterotermes aureus,* associated with circular grids that were treated with termiticides. Termites were collected monthly from 9 plots located at the Santa Rita Experimental Range (Pima Co., AZ) over a 3-year period. Plots were equally and randomly assigned to three treatments: a control and two insecticide treatments of either fipronil (Termidor®, BASF) or chlorfenapyr (Phantom®, BASF). Within the chlorfenapyr (Phantom®) treatment, we saw a significant increase in termite foraging populations after termiticide application. This effect showed a spatial pattern in which more termites were found near the center of plots. However, the number of *G. perplexus* collected in the fipronil (Termidor®) plots was reduced with respect to controls. This reduction in the number of termites also showed a spatial distribution with the decrease in termite numbers being stronger near the center of plots within the treated zone.

[1] University of Arizona, Tucson, AZ 85721. Email: pbaker@ag.arizona.edu
[2] Corresponding author

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INTRODUCTION

Worldwide, subterranean termites offer significant ecological services as part of the detritus cycle. However, some species can also cause extensive damage to wooden structures as well as wood and cellulose products in temperate and tropical areas. In fact, the combination of damage and control/repair costs attributed to subterranean termites in the United States alone was estimated to cost consumers at least $US 1.5 billion (Su and Scheffrahn 1990).

Non-structure invading termites can be associated with, and occur in, the same areas as economically important termites. As a result, pest management actions might be inadvertently taken against benign or even beneficial termites. This is true for the tube building termite *Gnathamitermes perplexus* (Banks), which belongs to the family Termitidae and is found in the desert Southwest including southern Arizona, southern Nevada, southern California, far-western Texas, and northern Mexico (La Fage 1976; Jones and Nutting 1979). Its primary role in the ecosystems it inhabits is as a decomposer, feeding on a variety of cellulose materials. *G. perplexus* builds above-ground, sheet-like foraging shelters made of soil particles cemented together with saliva. Under the shelter of these structures, it eats away the weathered portion of dead wood and other cellulose materials, sometimes consuming desert shrubs and grasses completely and leaving only hollow earthen tubes (Light 1934). *G. perplexus* plays an important role in the Sonoran Desert ecosystem by aerating the soil horizon (La Fage 1974). It has been estimated that this termite species moves over 500 kg/ha of soil every year, and in the process, it changes the soil composition by incorporating more clay into it and enriching it with carbon, nitrogen and other nutrients (Nutting *et al.* 1987). Although *G. perplexus* workers might be similar to those of other species, they are easily distinguished from the co-occurring species *Heterotermes aureus* (Snyder) by their size and coloration (Baker and Marchosky 2005). *G. perplexus* is not a serious economic pest as it does not cause structural damage. Homeowners may on occasion encounter their mud sheets covering the remains of dead cacti in contact with the soil or at the base of trees or fence posts.

Since *G. perplexus* generally does not invade structures, this species is essentially a non-target organism for the application of termiticides. Although the impact of commercial termiticides on *G. perplexus* has not been studied previously, it is likely that their populations and foraging activity could be negatively impacted by many of the pest management actions directed to other termite pest species. This would result in the loss of the valuable ecological services that this species provides.

Here we report the measurements of *G. perplexus* foraging populations collected over 3 years as part of a study on the effects of fipronil (Termidor®) and chlorfenapyr (Phantom®) as termiticides applied to soil. Both insecticides have a broad spectrum of action, are non-repellent termiticides and their active ingredients are of relatively recent discovery. Fipronil is a neurotoxin that acts by blocking GABA-regulated chloride channels, while chlorfenapyr is a pro-insecticide that uncouples oxidative phosphorylation by disrupting the production of ATP (Pegido and Rice 2006). They are registered for termite management and their use for this purpose is widespread. The active ingredients of both termiticides are also found in many other insecticide formulations due to their relatively generalized modes of action. Besides evaluating the effects of these insecticides on termite density, we were also able to make some observations on the temporal variation of *G. perplexus* foraging activity.

MATERIALS AND METHODS

Field Site

The study was conducted at three distinct locations at the Santa Rita Experimental Range ≈ 40 km south of Tucson, Arizona, USA (elevation 984 m) (Baker *et al.* 2010). This area was previously utilized for foraging studies by Haverty and Nutting (1975) and described by Jones *et al.* (1987). The results reported here are part of a larger study designed to evaluate the effects of two insecticides, fipronil and chlorfenapyr on foraging termite populations of the economically important termite *H. aureus.* We first surveyed the research area looking for places heavily infested with *H. aureus*. Then, several transects consisting of 10 to 15 termite collecting stations were placed in a 1.03 km^2 area during the spring of 2004. Each collection station consisted of three corrugated cardboard rolls (0.04 x 1.0-m strip of CR 30 x 250 B-flute SF cardboard, Tucson Container Corp, Tucson, Arizona, USA) wrapped around a piece of ash wood (*Fraxinus sp.*) (7.5 x 2.5 x 1.25-cm). Cardboard rolls were placed within a piece of 0.15-m diameter x 0.15-m long PVC pipe and covered with a concrete brick (20.3 x 15.2 x 2.5-cm). The surrounding collection stations were visually inspected approximately every four weeks for the presence of termites. Once a station was determined to contain several hundred termites, a circular feeding grid was constructed around it. Each grid consisted of 50 collection stations surrounding the central infested station and placed equidistantly (≈ 3.13-m apart) along the circumference on five circles or rings with increasing radii of 1.5, 2.0, 4.0, 7.0 and 10.0m; respectively. Each ring was labeled A-E with the center station designated by X (see Fig. 1). The three cardboard rolls contained within each of the 51 collecting stations in each experimental grid were labeled to link them to the corresponding feeding station. Three research grids (replicates or separate sites) were established for each insecticide treatment. In some cases, it took several months (July 2004 through March 2005) to establish a research grid due to temporal termite population fluctuations. Visual inspections of all

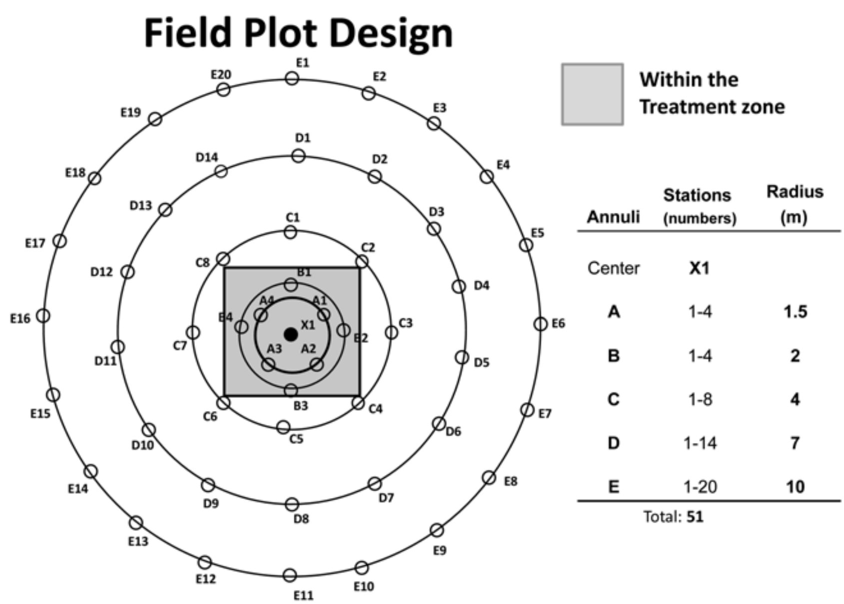

Fig. 1- Circular field plot design used where termite stations were placed in a grid formation by concentric rings of increasing size.

stations were conducted monthly from the end of 2004 thru mid-2008 and termites were counted in each station. Termite-infested cardboard rolls inside stations were replaced with new ones and were taken to our laboratory where the termites within were counted.

Termiticides

The termiticides chlorfenapyr (Phantom® 2 SC) and fipronil (Termidor® SC) (BASF Corporation), were applied at the rate of 0.125 and 0.06 on November 14, 2005, and September 19, 2006, respectively to each of 3 plots. Control plots (n=3) received only water on November 14, 2005. The applications were made by Johnston's Pest Solutions, a licensed pest control company. Applications were made by the standard application procedure of rod and trench at 4 gallons per 10 liner foot per trench between the B and C rings (see Fig. 1). The results presented here reflect only the *G. perplexus* counts.

In general, the experimental design reproduces the effect of using these termiticides as a barrier treatment in an area where termites are already present. The grid design employed was especially designed to determine the spatial magnitude termiticide effects. Since the termiticides used are non-repellent, termites are expected to come in contact with treated soil and die. Changes in the distribution of termites can also be the result of indirect effects of the termiticide treatment on termite foraging patterns. For example, if none of the termite foragers traveling in a given direction returns, this might discourage further movement in that direction.

Data Analysis We used the total termite count for each station as a starting point for statistical analyses. This consists of the sum of the termites visually estimated inside each station in the field and those counted from inside cardboard rolls. Termite activity was recorded before and after treatment for all plots. In order to use the control grids as a baseline representing the natural termite activity, we averaged the termite counts of the 3 replicate grids for each treatment by month. Then, we calculated the difference between treated and control plots (difference = termiticide - control) for each month for both termiticide treatments. We used 2-sample t tests to compare the mean difference between control and treatment grids before and after treatment. This analysis was performed for whole grids, and subsets of them including the treatment zone (the center station or X and the A and B rings), and each one of the rings outside it (C, D and E). A square root transformation was used whenever necessary to improve the normality and homogeneity of variance of the data. If homogeneity of variance could not be achieved through transformation, a Welch's ANOVA was calculated instead. Since collections for fipronil and chlorfenapyr grids were done at different times, their data were compared to different subsets of the monthly data for the controls.

Additionally, we looked for spatial patterns in *G. perplexus* density after treatment. We considered termite density as the average number of termites per station within a given area. Both occupied and unoccupied stations were considered for this calculation. If the effects observed are caused by the termiticides, we would expect them to be stronger inside the treatment area and decrease with distance from it. We compared the mean termite densities of the different areas within plots with non-parametric Kruskal-Wallis tests. This test was used because termite density data was highly skewed due to the high frequency of low termite counts. Whenever significant differences were found, a pair wise Mann-Whitney test with a Bonferroni correction was calculated.

RESULTS AND DISCUSSION

Both *H. aureus* and *G. perplexus* foragers were collected in monitoring stations at all nine sites. However, the two species never occupied the same station at the same time. In addition, *G. perplexus* foragers represented only the 6.94% (58,854 out of 847,685 in total) of the termites collected from all sites. Although the average number of *G. perplexus* in control plots for any given month was low (124.04 individuals per plot), a few explosive increases in foraging population numbers were observed in the fall (Fig. 2). It might be relevant that these population increases happened in the late summer, when soil moisture is higher as a result of rainfall associated with the monsoon. The highest number of *G. perplexus* found in a single plot (2,565 individuals) was collected in November of 2005 from one of the fipronil plots before treatment. The number of stations within each plot occupied simultaneously by *G. perplexus* was also considerably low. In control plots, which represent natural termite populations, averages of 3.3 stations out of the 51 that make up the grid were occupied any given month. As with total termite counts, station occupancy was quite variable and it was particularly low during the winter months. The maximum number of stations occupied within one plot simultaneously was 25, but this was a unique event.

Strong variation from one month to the next was evident and was likely related to weather or other environmental abiotic factors such as soil moisture, since the patterns of *G. perplexus* fluctuation where similar among untreated plots (Fig.2). This suggests a factor that affects all *G. perplexus* populations in the region simultaneously. Soil moisture and temperature have been previously shown to be determinant on the foraging activity of *G. perplexus* (La Fage *et al*. 1976). Taking these natural fluctuations into account, we considered that a decrease in termite activity after treatment does not necessarily indicate an effect caused by the termiticides. Instead, we looked at changes in termite activity with respect to termite counts from the control plots. If a termiticide had an effect on termite activity, we would expect to see greater differences between treatment and control plots after application.

When whole experimental grids are considered, the number of *G. perplexus* collected in the chlorfenapyr plots significantly increased with respect to the controls after treatment. Although an increase in the number of foraging *G.*

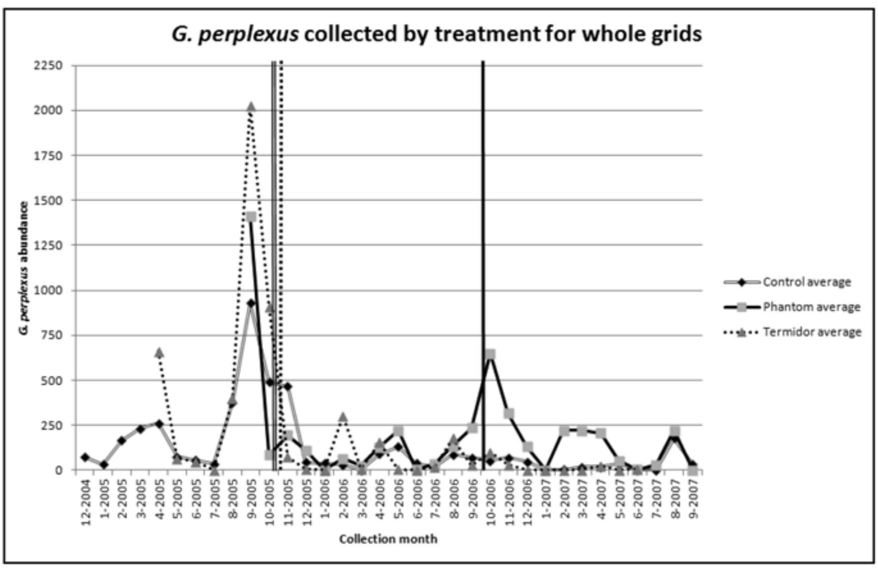

Fig. 2 – Average number of *G. perplexus* workers collected in all stations by month. The dates of application of the two treatments are marked with vertical lines of the corresponding pattern.

Table 1 – Mean difference in the number of termites collected between chlorfenapyr (Phantom®) designated plots and untreated controls before and after treatment. Mean negative values indicate lower termite abundance with respect to control plots. Tests were performed for the whole experimental grid as well as subsets. In some cases, a cubic root transformation was used to improve the normality and homogeneity of variance of the data.

Differences between the numbers of *G. perplexus* collected from chlorfenapyr and control grids

		Before treatment		After treatment				
Area considered	N	Mean	± SE	Mean	± SE	test statistic	p	Test performed
Treatment zone	25	12.4	4.9	36.4	23.2	t=-0.18	0.8619	2-sample t test on cubic root transformed data
C ring	25	14.3	15.7	25.0	12.7	t=0.32	0.7501	
D ring	25	-9.8	30.1	26.2	15.1	t=-1.37	0.1831	
E ring	25	-1.1	19.4	45.0	20.3	t=-1.84	0.0782	
Whole grid	25	-23.0	46.1	132.6	50.5	t=2.28	0.0328+	2-sample t test

+ Differences are significant but in the opposite direction than would be expected (The number of termites in clorfenapyr grids increases with respect to controls after treatment) .

perplexus was observed in all the rings that make up a plot, no significant differences were found when C, D, and E rings were considered independently (Table 1). This positive effect of chlorfenapyr on *G. perplexus* foraging populations could be explained by a decrease in the populations of the competing termite *H. aureus*. It has been established that these two species show interference competition and even aggression in the wild (Jones and Trosset 1991). We will consider this possibility in future work including data on *H. aureus* populations.

When we compared the number of termites per station in different zones of the experimental grid, a spatial pattern was apparent. After treatment with chlorfenapyr, termite numbers were higher in the treatment zone and they decreased with further distance from it. This kind of pattern was not present before application (Fig. 3). Although differences in *G. perplexus* density among different zones were not significant (Kruskal-Wallis test, H=5.091, Hc=6.469, p=0.165), this certainly reinforces the idea that the application of chlorfenapyr was somehow advantageous for these termites.

The plots treated with fipronil showed a rapid decrease in *G. perplexus* foraging populations immediately after treatment. This change can be clearly appreciated when only the treatment zone is considered (Fig 4). Inside it, *G. perplexus* numbers dropped to zero after treatment and did not recover afterwards. A significant decrease with respect to the controls was observed when whole plots where considered, as well as with the treatment zone (Table 2). For the C-ring, directly next to the treatment zone, results were suggestive of an effect although not significant (p=0.0614, see Table 2). Although *G. perplexus* were not completely eliminated from the outermost area of the

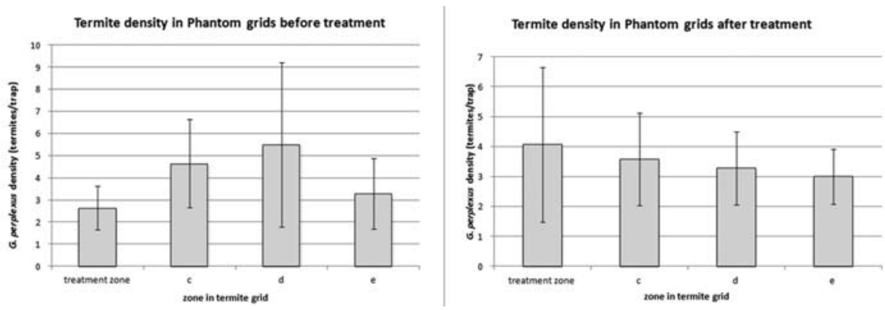

Fig. 3 - *G.perplexus* density (workers per station) before and after treatment with chlorfenapyr (Phantom®). Results are shown for different zones within the grid: the treatment zone and each of the rings external to it. The standard error of the mean was used for error bars.

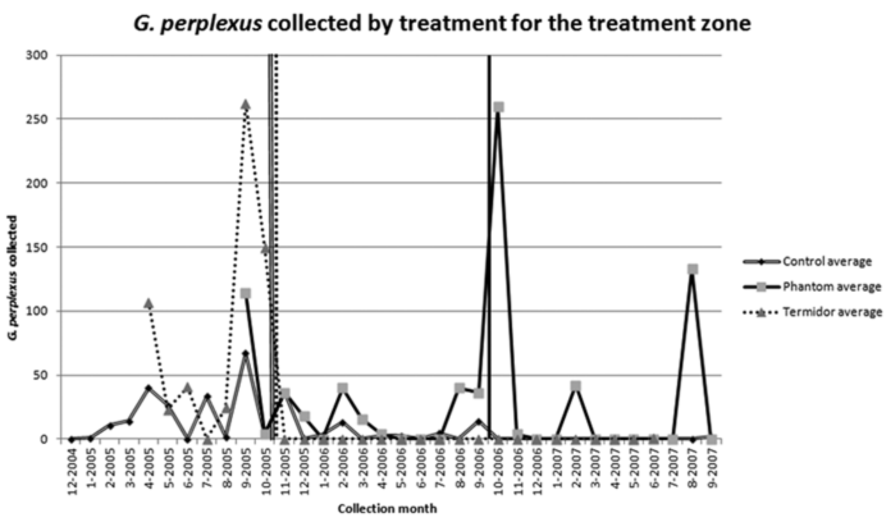

Fig. 4 - Average number of *G. perplexus* workers collected by month for the stations inside the treatment zone (A , B rings and X) for each treatment. The dates of application of the two treatments are marked with vertical lines of the corresponding pattern.

grid (the E ring), their numbers decreased significantly with respect to the controls (Table 2).

A spatial pattern for the density of *G. perplexus* was also evident as a result of fipronil application (Fig. 5). After treatment, termites were almost entirely absent from the treatment zone and the C ring. In contrast, the D and E rings had considerable numbers of termites present. The E ring, which is external to

Table 2 - Mean difference in the number of termites collected between fipronil (Termidor®) designated plots and untreated controls before and after treatment. Mean negative values indicate greater termite abundance with respect to control plots. A cubic root transformation was used to improve the normality and homogeneity of variance of the data. Also, a Welch's ANOVA was used when the data lacked homogeneity of variance after transformation.

Differences between the numbers of *G. perplexus* collected from fipronil and control grids

		Before treatment		After treatment				
Area considered	N	Mean	± SE	Mean	± SE	test statistic	p	Test performed
Treatment zone	18	62.2	31.0	-7.0	3.3	F=7.70	0.0284*	Welch's ANOVA on cubic root transformed data
C ring	27	65.0	50.9	-8.7	4.2	F=5.07	0.0614	
D ring	27	-20.6	17.3	-23.3	15.1	t=1.20	0.2416	2-sample t test on cubic root transformed data
E ring	27	162.5	91.4	18.8	17.5	t=2.10	0.0457*	
Whole plot	27	269.0	156.9	-17.0	26.5	t=2.06	0.0496*	

* Number of termites collected decreased significantly with respect to controls after fipronil treatment.

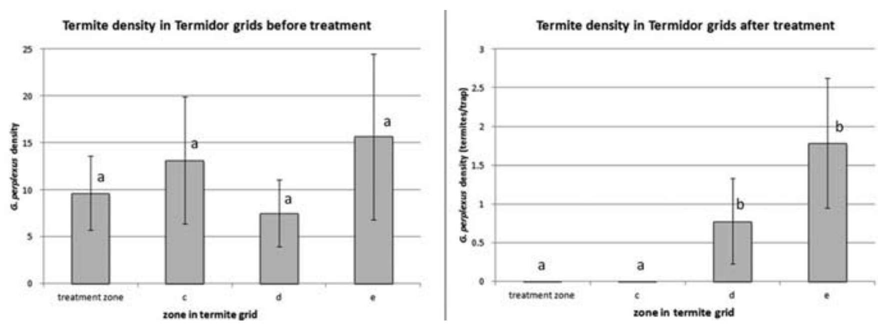

Fig. 5 - Termite density (workers per station) before and after treatment with fipronil (Termidor®). Results are shown for different zones within the grid: The treatment zone and each of the rings outside. The standard error of the mean was used for the error bars. Different letters are used to show significant differences in medians when these are present (determined with post-hoc Mann-Whitney tests with a Bonferroni correction). Notice the different scales used in the vertical axes of the graphs.

all others, had the highest density of *G. perplexus,* although this was still lower than the termite density in the same area before treatment. The differences between the numbers of termites collected in different rings after treatment were significant (Kruskal-Wallis test, H=10.89, Hc=31.57, p=0.0123) and they delimited two distinct areas in the termite grid (Fig.5). This pattern could be explained by the capacity of fipronil for horizontal transfer between termites. In trials performed with the subterranean termite *Reticulitermes hesperus*, termite mortality was delayed up to one week at low doses which allowed for the transfer of fipronil to termites that were not directly exposed (Saran and Rust 2007). In the same study, it was determined that termites could transport fipronil to a distance of at least 3 meters from the exposure site. Our results suggest that fipronil could have an effect on *G. perplexus* at 6 meters or more from the site of application.

In summary, these results show that fipronil has a strong negative effect on *G. perplexus* when applied as described. Applications of this termiticide would affect *G. perplexus* in the direct proximity of structures, and would also have an effect on termite populations in the surrounding areas, although to a lesser extent. It remains to be tested whether this is a direct result of the insecticidal activity of fipronil or is caused by a disruption of the normal movement patterns of *G. perplexus*. On the other hand, chlorfenapyr seems to slightly favor *G. perplexus* populations, probably as a result of the elimination of competing termites. In general, the use of chlorfenapyr would allow the persistence of *G. perplexus* and the preservation of the ecological services that this termite provides.

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