

The Influence of Fipronil on *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) Feeding Beyond Treated Plots

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ABSTRACT A small-plot field trial was conducted to examine the area of influence of fipronil at incremental distances away from treated plots on the Harrison Experimental Forest near Saucier, MS. Small treated (water and fipronil) plots were surrounded by untreated wooden boards in an eight-point radial pattern, and examined for evidence of termite feeding every 60 d for 1 yr after treatment. Circular areas of 0, 0.28, 1.13, 2.55, 4.52, 7.07, and 10.18 m² around the treated plots were installed to evaluate feeding damage by termites on the boards. The relationship between feeding damage to boards and area for each time interval was examined by using an exponential increase model. For both treatments and controls in nearly all periods examined, feeding was suppressed in the boards nearest to the treated plots, but increased exponentially as the area increased. Beginning 4 mo after treatment, treatment plots had lower proportions of boards with termite feeding evidence than control plots. Reduction in feeding was the only influence of fipronil observed beyond the treated plots.

KEY WORDS fipronil, foraging behavior, *Reticulitermes*, termite, termiticide

Termiticide transfer, or the movement of delayed-action nonrepellent (DANR) termiticides from exposed termites to naïve nestmates, has been the subject of much research over the past decade (Ferster et al. 2001; Thorne and Breisch 2001; Ibrahim et al. 2003; Shelton and Grace 2003; Hu et al. 2005; Shelton et al. 2005, 2006; Song and Hu 2006; Gautam and Henderson 2011; Shelton 2012). Essentially, termites encountering lethal (but slow-acting) doses of soil termiticides (donors) move to other parts of the nest where naïve (recipient) termites contact them (Haagsma and Rust 2007) and become exposed to the insecticide, ultimately leading to the death of the recipients (Ferster et al. 2001, Thorne and Breisch 2001). This raises many questions, such as how many donors are necessary to move enough insecticide to cause mortality, although attempts have been made to quantify the amount of material moved on individual termites (Rust and Saran 2006, Shelton et al. 2006).

In some transfer studies, termite mortality varied considerably with relatively low mortality in the recipient populations (Shelton et al. 2006, Rust and Saran 2008, Shelton 2009). However, most of the laboratory data supported the idea that transfer could occur under very specific (i.e., laboratory) conditions (Ferster et al. 2001, Thorne and Breisch 2001, Ibrahim et al. 2003, Shelton and Grace 2003, Hu et al. 2005,

Shelton et al. 2006, Song and Hu 2006, Haagsma and Rust 2007; Rust and Saran 2008, Shelton 2009, Gautam and Henderson 2011, Buczowski et al. 2012, Gautam et al. 2012). It should be noted that there is a limited amount of termiticide that can be carried by an individual termite (Rust and Saran 2006, Saran and Rust 2007), as well as limitations on the distance to which donor termites have been observed to move (Su 2005, Saran and Rust 2007).

Field studies with DANR products have given conflicting results. Potter and Hillery (2002) examined fipronil and imidacloprid treatments of infested barns and other structures, suggesting transference. Osbrink et al. (2005) found no evidence of imidacloprid-caused mortality at monitoring stations placed near imidacloprid treatments in New Orleans. Treatments to trees with imidacloprid foam suppressed, but did not eliminate, Formosan subterranean termites in nearby monitors (Osbrink and Lax 2003). However, Parman and Vargo (2010) found that imidacloprid treatments to homes resulted in the authors being able to detect only 3 of 12 previously treated termite colonies 2 yr after treatment. Ripa et al. (2007) working with fipronil in Chile found a reduction in activity only in monitoring stations set within 2 m of the treatments. Vargo and Parman (2012) surrounded houses with monitors and examined the genetic colony identification of the termites, both before and after a treatment with fipronil. Their study showed that all colonies within 6 m of the treatments vanished and did not return during 3 yr of monitoring posttermiticide application.

This article reports research results and their interpretation. Mention of a proprietary product does not constitute an endorsement or recommendation by the USDA Forest Service.

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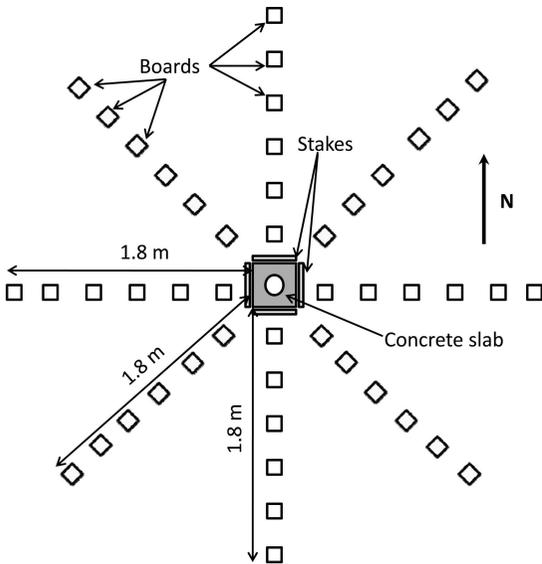


Fig. 1. Design for each plot. Center square is a concrete slab (53 by 53 cm); smaller squares are boards (14 by 14 by 2 cm) placed at 0.3, 0.6, 0.9, 1.2, 1.5, and 1.8 m from the concrete slab edge. Rectangles are stakes (45.5 by 4.5 by 2 cm) placed in the ground lengthwise along the edge of the concrete slab in the four cardinal directions. Figure is not to scale.

These field studies have left the impression that DANR termiticides have an area of effect surrounding treatments. This helps explain the lack of control plot termite feeding activity in the original U.S. Department of Agriculture Forest Service fipronil studies (Kard 2001). However, other theories have also been proposed to explain these data, such as the idea that this lack of feeding activity was the result of large areas of coverage with fipronil leading to direct exposure of the termites (Peterson et al. 2006, Vargo and Parman 2012).

Although not truly measuring transfer, one method of looking at the ability of a compound to extend its effect beyond a treated area is to measure its ability to prevent termite feeding at monitoring stations surrounding a treatment. This study examines whether a small (0.19-m²) DANR termiticide (fipronil) treatment can influence termite feeding in nearby monitors.

Materials and Methods

Untreated southern yellow pine (*Pinus L. spp.*) boards (14 by 14 by 2 cm) were placed in an asterisk pattern (Fig. 1) around a concrete slab that covered soil treated with either a termiticide (fipronil) or water only (controls). These two treatments were each replicated five times randomly among ten 4.3 by 4.3-m square areas in an open field on the Harrison Experimental Forest, Harrison County, MS. The presence of termite damage to the boards was collected as binary data. To determine if termites had contacted the treatment area, a set of stakes (each 45.5 by 4.5 by 2 cm,

southern yellow pine) were placed parallel to the soil surface (≈ 2.5 cm into the soil) at 0 m (edge of the treatment frame) along all four sides of the frame (Fig. 1). There were eight boards (subsamples) at each distance (0.3, 0.6, 0.9, 1.2, 1.5, and 1.8 m) per replicate. Distances were considered radii for circular areas surrounding each plot. Therefore, there were five replicates for each area for regression. Regression was used to look for a relationship between termite feeding evidence and area surrounding treated and untreated plots by using an exponential increase model.

Initial Plot Layout. By using a hoe, plots were cleared to mineral soil at the center of each 4.3 by 4.3-m replicate area. A board was placed at the center temporarily as a placeholder until the actual treatments were implemented. A 53.34 by 53.34-cm wooden frame was placed around the board to represent the edges of the eventual concrete slab, and by using a compass, the boards were aligned centered on a 0.3-m spacing from the edge of the frame (i.e., at 0.3, 0.6, 0.9, 1.2, 1.5, and 1.8 m) to the N, NE, E, SE, S, SW, W, and NW of the plot, respectively (Fig. 1). A hoe was used to clear the area beneath each board. There were 48 boards (plus four stakes) per replicate; therefore, 480 boards (plus 40 stakes) were read at each inspection interval.

Reading. Beginning 6 mo after initial plot installation, all boards were examined once every 60 d, and boards with termites or evidence of their feeding were replaced at each reading. Readings continued until the majority of boards showed evidence of termite feeding (a threshold of 75%), including the center boards for all replicates. After this threshold was reached, the treatments were implemented on the next visit. Readings continued every 60 d for 1 yr after the treatment application to soil, replacing boards that showed evidence of termite feeding.

Treatment. Plot size and treatment methods used were similar to those used by Kard (2001). At 246 d, the center 0.19 m² of each replicate area was again cleared to mineral soil. The wooden 53.34 by 53.34-cm concrete frame was replaced (if damaged) around the center board. The board as well as roots, rocks, and debris were removed from the treatment area. A square metal treating frame (43.18 by 43.18 cm) was placed in the center of the wooden frame. The soil area within the treating frame received 757 ml (to give an application volume equivalent to 3.79 liters per 0.93 m² [1 gal per 10 feet²], as required by the treatment label) of either water (controls) or fipronil (a 0.125% (AI) solution of Termidor SC [BASF corporation, Research Triangle Park, NC], following label instructions) by using a watering can. Treatment for each replicate was determined by a coin toss. The treated soil was covered with a vapor barrier and an 11.43-cm-long 10-cm-diameter PVC pipe was centered vertically on the vapor barrier. The area within the wooden frame was filled with concrete to form a slab roughly 53 by 53 cm with the PVC pipe in the center. The next day, the vapor barrier within the PVC pipe was cut out of the bottom of each pipe and a wooden block (8.9 by 6.4 by 3.8 cm) placed inside, and finally,

Table 1. Regressing proportion of termite-damaged boards (y) on area (x; as means), using the model $y = a + be^{-x}$

Day	Treatment	Month	a^a	b^a	r^2	P^b
179	Control	Jan. 2011	0.834 ± 0.014	0.029 ± 0.028	0.175	0.35022
	Fipronil	Jan. 2011	0.949 ± 0.004	0.006 ± 0.009	0.089	0.51592
246	Control	Mar. 2011	0.480 ± 0.006	-0.039 ± 0.011	0.715	0.01655
	Fipronil	Mar. 2011	0.652 ± 0.006	-0.006 ± 0.012	0.042	0.65780
Treatment Implemented						
310	Control	May 2011	0.438 ± 0.013	-0.106 ± 0.026	0.770	0.00947
	Fipronil	May 2011	0.520 ± 0.009	-0.081 ± 0.018	0.796	0.00690
368	Control	July 2011	0.806 ± 0.014	-0.069 ± 0.028	0.543	0.05857
	Fipronil	July 2011	0.611 ± 0.023	-0.301 ± 0.046	0.896	0.00123
429	Control	Sept. 2011	0.882 ± 0.013	0.087 ± 0.027	0.671	0.02427
	Fipronil	Sept. 2011	0.681 ± 0.026	-0.360 ± 0.053	0.902	0.00105
492	Control	Nov. 2011	0.696 ± 0.020	-0.193 ± 0.040	0.824	0.00474
	Fipronil	Nov. 2011	0.529 ± 0.012	-0.059 ± 0.023	0.557	0.05404
554	Control	Jan. 2012	0.521 ± 0.019	-0.219 ± 0.039	0.865	0.00239
	Fipronil	Jan. 2012	0.449 ± 0.019	-0.350 ± 0.040	0.940	0.00030
619	Control	Mar. 2012	0.620 ± 0.026	-0.297 ± 0.054	0.860	0.00264
	Fipronil	Mar. 2012	0.438 ± 0.028	-0.328 ± 0.057	0.867	0.00229

Readings before the implementation of treatment (on day 246) are separated into plots that will become controls and those that will become treated plots. Model parameter a is a constant derived from the data, b is a coefficient derived from the data, and e is a mathematical constant (≈ 2.71828).

^a Coefficients are presented as means \pm SEM.

^b P values < 0.05 represent significant models for the feeding data, not comparisons between treatment and controls.

a PVC cap was placed on the pipe. From this point forward, the wooden blocks were read for each replicate.

Species Identification. On the final two readings of the study, 554 and 619 d, single infested boards were collected from individual replicates and returned to the laboratory. Termites were extracted from the boards and preserved in 90% ethanol before identification by using soldier morphology characters as described by Lim and Forschler (2012).

Data Analysis. For the two pretreatment readings (179 and 246 d), replicates were separated into those that would eventually become treated or control plots. As there is an assumption that the influence of fipronil (if any) will spread outward from the point source (plot center) in a circular fashion, each distance of boards was considered a circle (thus making each distance into the radius of an area surrounding the plot). Because areas are being considered, the proportion of boards with evidence of termite feeding in each area was calculated for each replicate from the raw data and was regressed on area size, by using an exponential increase model (Tablecurve 2D; Systat Software, Inc. 2002).

Results and Discussion

The first reading was made 179 d after the plot installation (Table 1). At this time, all center boards and 90.2% of the surrounding boards showed evidence of termite feeding. Treatments were initiated on the next visit (246 d), after reading the boards. Regressions of proportion of boards with evidence of termite feeding on area for each period separated by treatment are presented in Table 1. The proportion of boards with termite feeding at each period is presented in Figs. 2–5, including only the significant regression models. At 619 d (Fig. 5), two control replicates were lost because of wildlife activity, so there

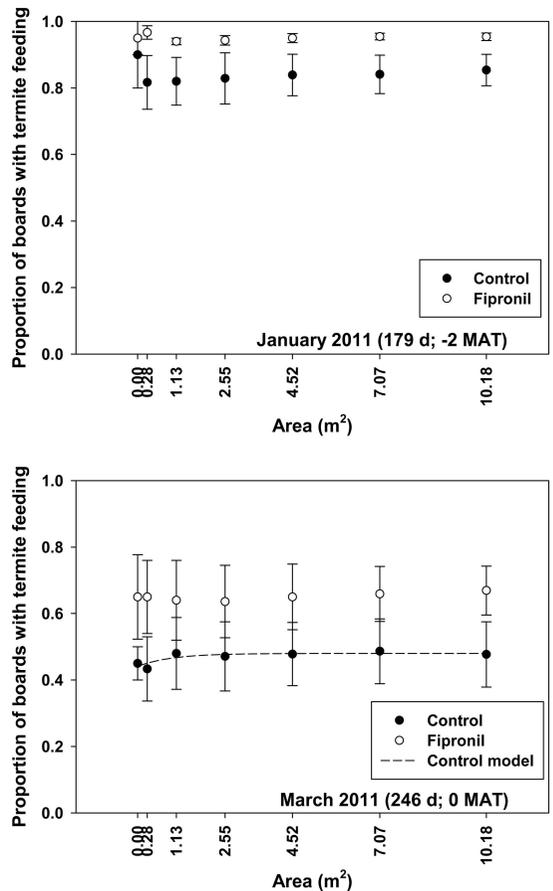


Fig. 2. Pretreatment proportions of boards with termite feeding (y) regressed on area (x) surrounding plots that will eventually become control and treated plots at 179 and 246 d. MAT, mo after treatment. Only lines of significant models are shown.

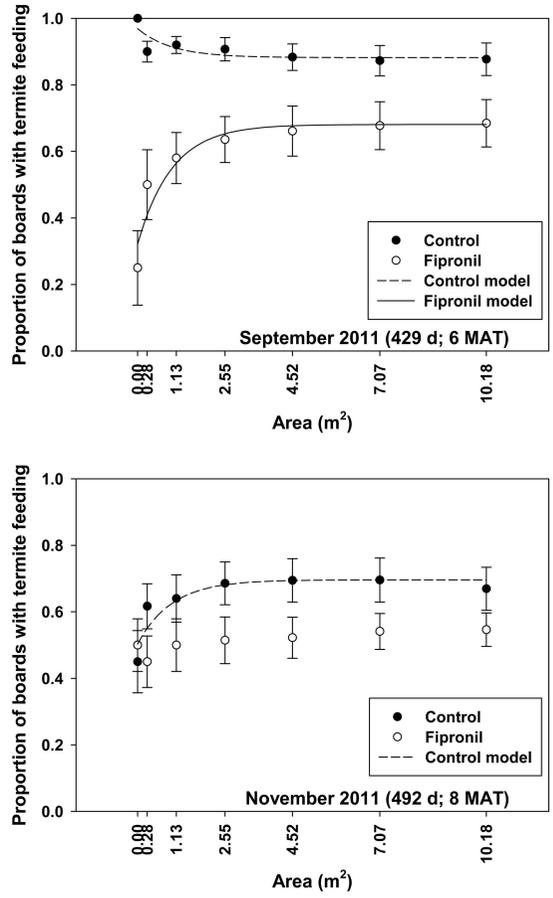
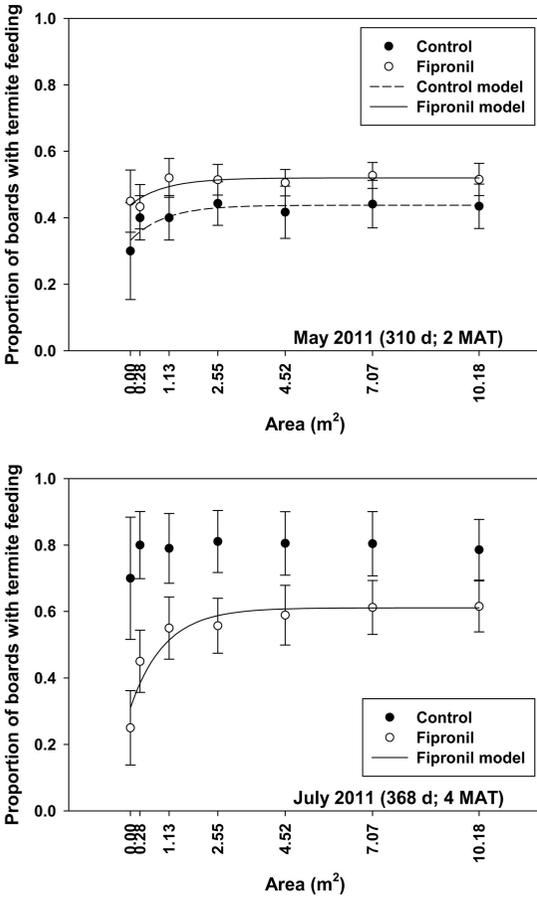


Fig. 3. Proportions of boards with termite feeding (y) regressed on area (x) surrounding both control and treated plots at 310 and 368 d. MAT, mo after treatment. Only lines of significant models are shown.

Fig. 4. Proportions of boards with termite feeding (y) regressed on area (x) surrounding both control and treated plots at 429 and 492 d. MAT, mo after treatment. Only lines of significant models are shown.

were only three control replicates for that day. Soldiers recovered were identified as *Reticulitermes flavipes* (Kollar) (Lim and Forschler 2012).

During the pretreatment periods and the first post-treatment reading, plots designated to become treated had a trend of greater proportions of boards with evidence of termite feeding than the controls (Fig. 2). This phenomenon reversed starting at 368 d, and continued to the end of the study (Figs. 3–5). The differences in proportion of boards with evidence of termite feeding between the controls and treatments represent the influence of fipronil on these monitors. Overall, these data are consistent with the idea of a suppression of termite feeding on boards placed around the fipronil-treated center plots, but not with feeding elimination.

The model $y = a + be^{-x}$ (Table 1), where y is the mean proportion of boards with termite feeding evidence, a is a constant derived from each data set, b is a coefficient derived from each data set, e is a mathematical constant (≈ 2.71828) that is the basis for natural logarithm, and x is area, describes the mean proportions of termite-damaged boards for both treat-

ments and controls throughout most of the periods, beginning with the second pretreatment reading. Therefore, the reduction in the proportion of boards with termite feeding in the three areas closest to the treatment had nothing to do with the treatment, and was probably an artifact of how termites responded to the experimental design. It is unlikely that this artifact was because of season, as it appeared throughout the study (note that at 429 d, the control data model was inverted; Table 1; Fig. 4).

There are at least two possibilities for this artifact. One possibility is that the artifact is because of the increased density of boards near the center of each plot (i.e., the smaller areas; Fig. 6), where there is more disturbance of the feeding termites during the readings. This contrasts with other studies on *Reticulitermes* Holmgren spp. disturbance, which have shown that feeding was not effected by disturbance at 2-mo intervals (Shelton et al. 2011). However, in that study, there was a greater distance between boards (1.52 m) than in the current study (0.3 m). Another possibility is that there may be more wood surface area available in the center of these plots than the termites

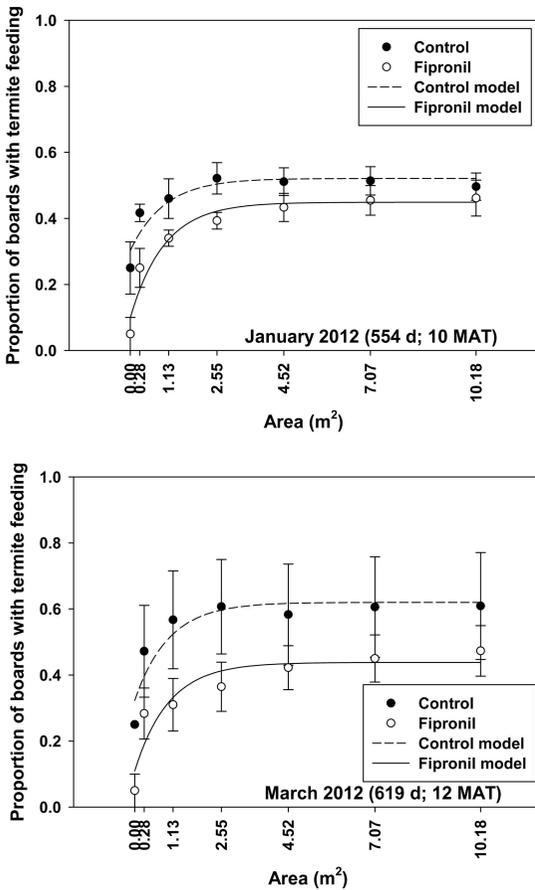


Fig. 5. Proportions of boards with termite feeding (y) regressed on area (x) surrounding both control and treated plots at 554 and 619 d. MAT, mo after treatment. Only lines of significant models are shown.

can effectively use over a 2-mo period. When termites are given a longer period to feed on boards, this artifact does not appear (see Fig. 2 and 179 d [6-mo feeding] and 246 d [2-mo feeding]). Feeding evi-

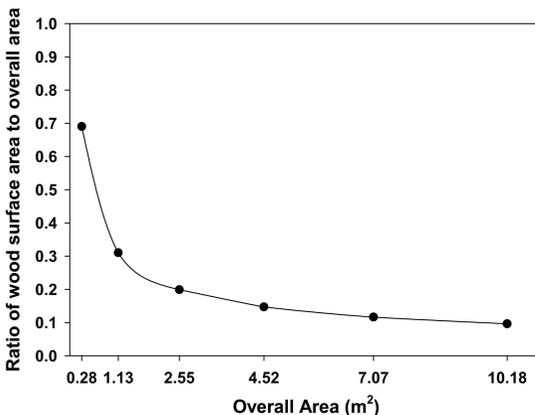


Fig. 6. Ratio of surface area covered with wood to overall area in all plots.

dence on boards at the 0-m² area (edges of the plots) did not reach zero in the treated or control plots (Figs. 2–5), suggesting that even at the edge of the treatment, termites are still actively feeding on the stakes and boards.

The current study’s results contrast with those obtained by Potter and Hillery (2002) and Vargo and Parman (2012) with fipronil against *Reticulitermes* spp. In their studies, feeding was suppressed almost completely (in 40 of 43 cases in Potter and Hillery 2002) near the fipronil treatments. In the current study, a (sometimes low) proportion of feeding was still observed at the 0-m² area from the treated plots throughout all periods. The main reason for this difference is probably size and type of application. In the articles by Potter and Hillery (2002) and Vargo and Parman (2012), perimeter treatments were made for entire structures. In the current study, treatments were only 0.19 m². The difference suggests that area of coverage is important for controlling termites with fipronil. Numerous termites may encounter a large treatment with fipronil (and possibly other nonrepellent termiticides), eventually controlling the population by direct action on large portions of the colony (Peterson et al. 2006, Vargo and Parman 2012), particularly if the main food source for the termite population is treated.

Comparisons between this study and the work of Kard (2001), the standard USDA Forest Service termiticide trials, are problematic. There is a difference in the application size compared with the overall size of the plots; for this, study application size was 1.86% of the overall area of the plot. For the USDA Forest Service trials, application size is 8% of the overall area of the plot. The USDA Forest Service trials use 10 replications in a grid pattern, with multiple concentrations in the same grid. This layout has changed in recent years (Peterson et al. 2006). The increase in relative untreated area in the current study means a lower probability of contact between termites and the treatment, making a direct comparison between these studies unfeasible.

On the initial reading (179 d; Fig. 2), there was evidence of termite feeding at ≈90% of the monitor boards. Data in Figs. 2–5 indicate that the termite feeding on the monitors was constant, as there were no periods where feeding evidence on the boards dropped to zero, as might be expected if colonies had been eliminated. However, the purpose of the study was to examine if small treated plots could reduce or eliminate feeding in nearby monitors, not to control specific colonies.

R. flavipes colonies occupy small foraging areas in forested locations, such that most termite collections 15 m apart are from different colonies (Vargo 2003). The current study was performed in a field surrounded by a forest. It is possible that colonies affected by the treatments were not the same as the colonies foraging at boards surrounding those treatments. In this case, however, the colonies affected by the treatment would have to forage in a limited area to have no contact with the boards that were

0.3 m distant or the stakes that were next to the treatment (0 m distant; Fig. 1). This seems unlikely, given how the boards and stakes surrounded the treatment areas (Figs. 1 and 6).

Su (2005) found that fipronil and thiamethoxam did not meet the criteria for liquid termiticide baits, as the distance of movement of exposed termites (*Coptotermes formosanus* Shiraki) was <5 m. In the current study, the maximum distance between monitors and treatments was <2 m.

In summary, small plots of fipronil-treated soil did not cause termite feeding to cease in any area surrounding the treated plots within a year after treatment. Future work should include a range of application sizes when examining the effects of termiticides on feeding with *R. flavipes*.

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