Effect of Fipronil on Subterranean Termite Colonies (Isoptera: Rhinotermitidae) in the Field

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ABSTRACT To assess possible colony-level effects of fipronil, a commonly used nonrepellent termiticide, we conducted a field study of eight houses in the Raleigh, NC, area with infestations of the eastern subterranean termite *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae). We installed an extensive grid of in-ground monitoring stations on each property (mean = 68.6 monitors per property) and collected samples from these stations as well as from mud tubes in the structure and wood debris in the yard for ≈6 mo. We genotyped all samples by using microsatellite markers to identify the number and locations of colonies present on each property. Houses were treated with either a full treatment ($n = 5$) or exterior/localized interior treatment ($n = 3$). After treatment, the monitors were checked monthly for 3 mo and then quarterly for 3 yr to track the fate of colonies. Wood debris in natural areas was checked semiannually for 3 yr. All 11 of the treated colonies (those attacking structures) disappeared within 90 d of treatment and were not found again. These colonies were presumed to be eliminated. In contrast, 60% of untreated colonies (those located >6 m from the foundation wall at the time of treatment) continued to persist throughout the study, as did 25% of the likely treated colonies (those occupying monitors 0.5 m from the foundation wall where the treatment was applied). Our results provide strong evidence for potent colony wide effects of fipronil on subterranean termites leading to colony suppression and likely colony elimination under field conditions.

KEY WORDS *Reticulitermes flavipes*, *Reticulitermes hageni*, colony suppression, colony elimination, microsatellite

The application of liquid termiticides to the soil has been the mainstay of subterranean termite control for many decades. Traditionally, the goal of such a treatment is to create a continuous chemical barrier that excludes termites in the ground from entering a building. Over the past dozen years or so, nonrepellent liquid insecticides with delayed toxicity have come to dominate the postconstruction termiticide market in most regions of the United States due to their high efficacy and low rates of treatment failures (Anonymous 2002, Potter 2004). The main active ingredients in the new nonrepellents are fipronil (Termidor, BASF Corp., Research Triangle Park, NC), imidacloprid (Premise, Bayer Corporation Specialty Products, Kansas City, MO; several generic products), chlorfenapyr (Phantom, BASF Corp.), and chlorantraniprole (Altriset, DuPont Crop Protection, Wilmington, DE). There is growing evidence that at least some nonrepellent termiticides can be transferred among individuals within colonies (Ibrahim et al. 2003, Shelton and Grace 2003, Rust and Saran 2006, Tsunoda 2006, Haagsma and Rust 2007, Saran and Rust 2007, Spomer et al. 2008, Bagnères et al. 2009) and can have activity on termite colonies beyond the immediate zone of treated soil (Potter and Hillery 2002, Osbrink and Lax 2003, Ripa et al. 2007, Parman and Vargo 2010), possibly resulting in colony elimination.

Fipronil is a phenylpyrazole insecticide that disrupts central nervous system activity by blocking the γ-aminobutyric acid-gated chloride channel (Cole et al. 1993). It is highly toxic to termites, with a lethal $dose_{50}$ for the western subterranean termite, *Reticulitermes hesperus* Banks, of 0.2 ng per termite at 7 d (Saran and Rust 2007) and 1.33–1.39 ng per termite at 72 h for the Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Ibrahim et al. 2003). Based on the tunneling activity of *C. formosanus*, *Coptotermes heimi* (Wasmann) and the eastern subterranean termite, *Reticulitermes flavipes* (Kollar), fipronil is nonrepellent up to at least 100 ppm by weight of soil (Hu 2005, Remmen and Su 2005, Manzoor et al. 2009, Mulrooney and Gerard 2009), well above the 60 ppm rate of application. Studies have shown that once individual termites contact treated soil, they can move away from the treated area covering a distance of up to 2 m (Ripa et al. 2007, Saran and Rust 2007). In addition, exposed termites can transfer some of the

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toxicant to naïve termites; workers of *R. hesperus* and *R. flavipes* exposed to sand treated with <5 ppm can transmit lethal doses of fipronil to unexposed nestmates (Saran and Rust 2007, Bagnères et al. 2009), raising the possibility that fipronil treatments can affect entire colonies of subterranean termites.

Additional support for colony-level impact of fipronil comes from field observations indicating that fipronil can affect termites outside the immediate treatment zone. For example, Wagner (2003) reported a decline in termite activity in control blocks located nearly 2 m from treated blocks in the U.S. Forest Service termiticide tests. Similarly, Ripa et al. (2007) found activity of the eastern subterranean termite ceased in monitoring stations located 2 m from the site of fipronil treatment, and Potter and Hillery (2002) found that fipronil treatment affected termites of this same species occupying monitoring stations up to 4 m away from the treated area. However, there may be limitations on the distance over which fipronil treatment can affect subterranean termite colonies. Su (2005) reported that workers of the Formosan subterranean termite did not travel >5 m after contacting fipronil-treated soil. These authors suggested that any potential colony-level effects of fipronil would be limited to areas within 5 m of the site of treatment.

The true test of potential colony-level effects of fipronil requires that colonies exposed to field treatments be identifiable and followed over time. In many situations, the method of choice for identifying individual subterranean termite colonies and tracking their fate is genetic fingerprinting using microsatellite markers (Vargo and Husseneder 2009). Microsatellite genotyping has been used to track colonies and assess the efficacy of both baits (Vargo 2003, Aluko and Husseneder 2007, Husseneder et al. 2007) and liquid treatments (Parman and Vargo 2010). In a recent study, we used a combination of microsatellite genotyping and intensive monitoring to assess colony-level effects of imidacloprid treatment in the field over a 2-yr period in central North Carolina (Parman and Vargo 2010). Our results showed the likely elimination of up to nine of 12 treated *Reticulitermes* spp. colonies after imidacloprid treatment. Here, we use similar methods to determine the colony-level effects of fipronil treatment (Termidor) on subterranean termites infesting houses in North Carolina.

Materials and Methods

Study Houses. We selected eight residential, standalone houses with active termite infestations in and around Wake County, NC. These houses were part of a larger group of 20 houses studied by Parman and Vargo (2008) to investigate subterranean termite colony abundance and density on residential properties. The houses used in the current study averaged (± SD) 378.9 ± 253.7 m^2 (range, 182–789 m^2) and were situated on a mean lot size of 2,747 ± 2474 m^2 (range, 648-7568 m^2). They ranged in age from 6 to 49 yr (mean = 23.4 ± 17.2 yr).

We installed monitors around the structures and in the yard areas between 26 April 2001 and 9 April 2002. For any given property, monitors were installed in either a single day or over two consecutive days. Monitoring stations were constructed from 6 cm-wide polyvinyl chloride (PVC) tubing as described by Parman and Vargo (2008). In brief, monitors were 30 cm long with two pieces of 20-cm-long pine wood strips inside. The stations were inserted into prebored holes in the ground and covered at ground level with a PVC cap. Monitors were installed in two concentric rings around the structures. The inner ring consisted of monitors placed ≈0.5 m from the foundation wall and positioned 2–6 m apart. The outer ring of monitors was located ≈6 m outside of the inner ring, and in some cases, a third ring of monitors ≈6 m beyond the second ring was also installed. We installed an average of 68.6 ± 25.2 monitors on each property (range, 41–121). The locations of monitors around two representative houses are shown in Figs. 1 and 2.

Pretreatment Termite Sampling. We checked the monitors monthly for the presence of termites a mean of 7.1 ± 2.3 times over a period of 6.3 ± 1.1 mo (range, 4.9–7.9 mo) before treatment. In addition, we checked mud tubes and any infested wood in the structure for termite presence. We also checked other areas in the yard containing wood debris, stumps, wood piles, and other materials at least twice before treatment. Termite workers were collected from monitoring stations, mud tubes and wood debris; placed into vials containing 95% ethanol; and stored at −20°C until DNA extraction. We collected one final sample either the day of treatment or the day before treatment. We genotyped the pretreatment samples to determine colony affiliation as described below (see DNA Analysis for Termite Colony Affiliation) and then made maps of each property showing the locations of all active colonies present at the time of treatment (Figs. 1 and 2).

Treatment. Houses were treated using Termidor SC at the label rate of 0.06% by a commercial licensed pest management professional. Five houses received a full treatment following the instructions for postconstruction conventional termite treatment in which a continuous barrier of termiticide is applied to the interior and exterior perimeters, whereas three houses received the postconstruction exterior/localized interior structural termite treatment receiving a continuous exterior barrier with interior treatment around areas showing termite activity (Table 1). All treatments were done in accordance with the label, including trenching and rodding around foundation walls, as well as drilling slabs, hollow blocks, walkways, and porches, where appropriate.

Posttreatment Termite Sampling. We did extensive monitoring and sampling for 3 yr after treatment. After treatment, we checked all monitors and mud tubes for the presence of living termites weekly for 4 wk, after which time they were checked monthly for 6 mo and then quarterly for 2.5 yr. We followed this sample schedule closely in most cases, but in some cases it was shifted by a few weeks. The 90-d time period was represented by the sample collected closest to this

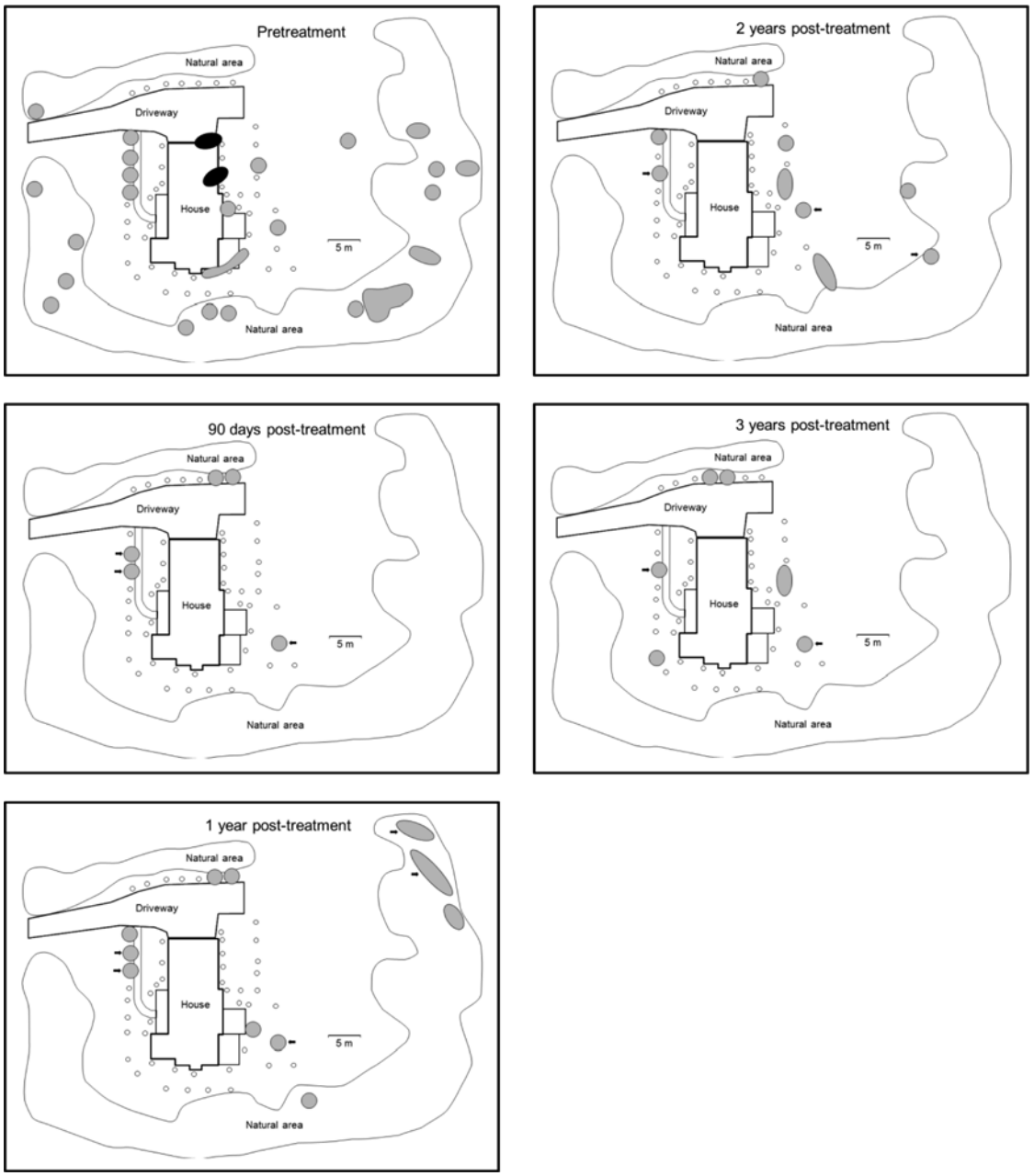

Fig. 1. Locations and observed foraging areas of *R. flavipes* colonies on property BW before treatment and at four time points after treatment. Small open circles represent in-ground monitoring stations. Gray larger circles and enclosed shapes represent colonies. The two dark elliptical shapes at the time of pretreatment depict foraging areas of two colonies that were infesting the structure. Black arrows indicate colonies present before treatment and redetected again at the later time points. This house received a full interior and exterior perimeter treatment.

data (range, 82–126 d after treatment). We sampled natural areas semiannually. Samples of any termites encountered were collected and held in vials containing 95% ethanol at −20°C until DNA extraction.

To gauge overall termite activity throughout the study period, we tallied the number of monitors with termites present for each property at the following time points: day of treatment (before termiticide application), ≈90 d after treatment, and ≈1, 2, and 3 yr after treatment. Analysis of these time points provided an indicator of termite activity just before treatment, 90 d after treatment, at which time all structures were free of termite infestations, and at yearly intervals.

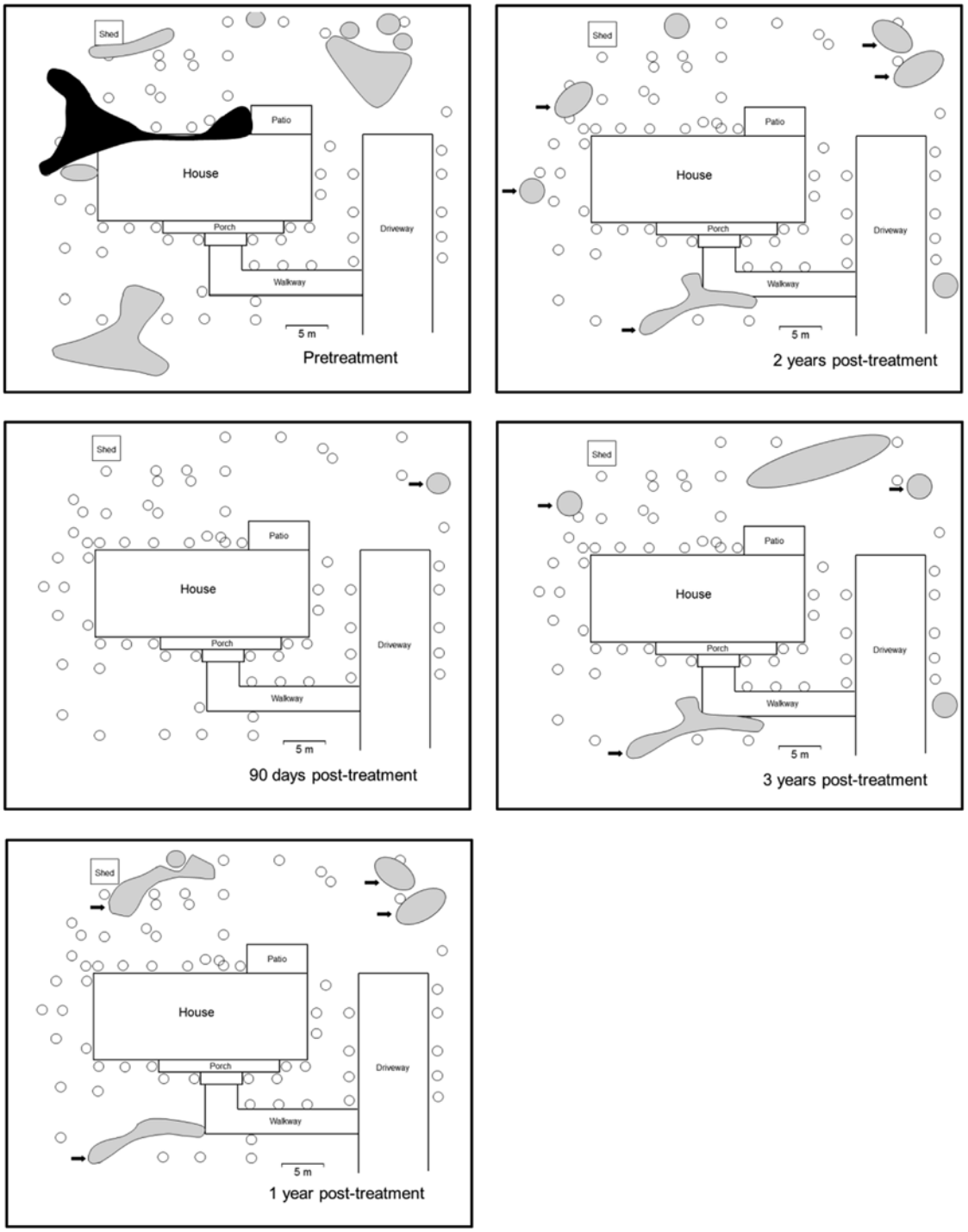

Fig. 2. Locations and observed foraging areas of *R. flavipes* colonies on property HI before treatment and at four time points after treatment. Small open circles represent in-ground monitoring stations. Larger circles and enclosed shapes in gray represent colonies not known to be infesting the structure. The dark irregular shape present in the pretreatment panel depicts a colony that was infesting the structure. Black arrows indicate colonies present before treatment and found again at the later time points. This house received an exterior/localized interior perimeter treatment.

Table 1. Characteristics of study properties and pretreatment sampling period

Property	Full interior and exterior treatment (FT) or exterior/localized interior treatment (EL)	Length of pretreatment monitoring period (d)	No. monitors	No. times sampled	No. samples collected	No. colonies in house	Additional colonies detected on property	Total no. colonies per ha
BW	FT	208	52	8	65	2	30	100.2
HI	EL	219	57	8	53	1	9	47.3
HU	FT	154	58	5	27	2	10	137.3
LR	EL	201	88	6	36	1	7	35.3
LO	EL	146	63	6	58	2	34	44.9
MN	FT	154	41	5	13	1	2	30.9
SB	EL	238	121	12	59	1	23	43.7
SM	EL	203	69	7	10	1	1	11.2
Mean ± SD		190.4 ± 34.4	68.6 ± 25.2	7.1 ± 2.3	40.1 ± 21.7	1.4 ± 0.5	14.5 ± 12.8	56.4 ± 41.4

DNA Analysis for Termite Colony Affiliation. We extracted DNA from whole termite bodies by using the Gentra PureGene kit (Gentra Systems, Inc., Minneapolis, MN) or the DNeasy kit (QIAGEN, Valencia, CA). In nearly all cases, we analyzed 10 individuals per sample (i.e., foragers present in the same monitoring station, piece of wood debris or mud tube), but in a small number of cases fewer than 10 individuals were present. Samples were first identified to species using the polymerase chain reaction (PCR)-restriction fragment length polymorphism method of Szalanski et al. (2003) on a fragment of the cytochrome oxidase II (COII) gene. The pretreatment samples were part of a previous study on colony densities around residential properties (Parman and Vargo 2008), where they were genotyped at 10 microsatellite loci: *Rf 24-2, Rf 21-1, Rf 15-2, Rf 6-1, Rf 5-10, Rf 1-3, Rs 16, Rs 33, Rs 62,* and *Rs 76.* We used the PCR conditions described by Vargo (2000) and Dronnet et al. (2004). After the pretreatment period, many samples were analyzed at only two loci (*Rf 24-2* and *Rf 15-2*), shown to be sufficient to determine colony affiliation in the study population (Parman and Vargo 2010), whereas others also were genotyped at *Rf 21-1.* PCR products were resolved on 6.5% polyacrylamide gels run on a 4300 automated DNA sequencer (LI-COR Biosciences, Lincoln, NE). Genotypes were scored by comparing the fluorescently labeled PCR products to size standards by using GeneProfiler version 3.56 (BD Biosciences Bioimaging, Rockville, MD).

We determined the colony affiliation of samples following the methods of Parman and Vargo (2008). In brief, the genotypes of the group of individuals comprising a sample were compared with all other groups collected from the same property by means of an exact test of genotypic differentiation by using GenePop on the Web (Raymond and Rousset 1995). Samples were considered to belong to the same colony if they shared all the same genotypes, and they did not differ significantly ($P > 0.05$) in genotype frequencies. The strong family structure of colonies in the study population, nearly all of which were founded by a monogamous pair of reproductives (Parman and Vargo 2008), together with the high genetic variability exhibited at *Rf 24-2,* with up to 36 alleles present in the local *R. flavipes* population (Parman and Vargo 2010), make colony identification straightforward and accurate.

Effect of Fipronil on Termite Colonies. To distinguish among possible treatment levels, all colonies present before treatment were placed into one of three categories as described in Parman and Vargo (2010). Treated colonies were those that were known to be infesting the structure, and because they were the targets of the treatment, are assumed to have been exposed to the applied fipronil. These colonies were occupying mud tubes on the foundation wall, present in structural elements, or both. Likely treated colonies were those occupying the inner ring of monitors located 0.3–0.6 m from the foundation wall where the treatment was applied. Although it is likely that these colonies were exposed to the treated soil, it is impossible to know for certain whether foragers from these colonies contacted the treated area. Finally, we considered colonies located in the outer ring of monitors or in the natural areas to be untreated colonies.

To determine the effect of the treatment on colonies, we tracked the fate of colonies in each of the three categories (treated, likely treated, and untreated) at four time periods: ≈90 d after treatment, between 90 d and 1 yr after treatment, 1–2 yr after treatment, and 2–3 yr after treatment. We recorded whether individuals from a colony were detected at each time interval. During the period between 90 d after treatment and the 1-yr mark, monitors were checked at least six times and natural areas at least twice. Between the 1- and 3-yr time points, monitors were checked four times per year and natural areas twice annually. In total, we genotyped 6,378 individuals from 1,037 samples (groups of works from monitoring stations, mud tubes, and wood debris).

Results

General Description of Colonies Present Before Treatment. Details of the numbers of colonies found in and around each structure before treatment are given in Table 1. In total, we detected 128 colonies present on the eight properties at the time of treatment, including those infesting the structures. Of these, the majority were *R. flavipes;* the only other species detected was *R. hageni,* of which we found nine colonies present (7% of the total) on four of the properties, and in all cases, these colonies were located in natural areas and were not found in any of the

Table 2. Effect of fipronil treatment on colonies of *R. flavipes* on eight residential properties in the Raleigh, NC, area

Colony type	Pretreatment count	Count 90 d posttreatment		Count 90 d to 3 yr posttreatment	
		Detected	Not detected	Detected	Not detected
Treated	11	0	11	0	11[a]
Likely treated	12	1	11	3	9[a]
Untreated	105	13	92	63	42

[a] Differed significantly from the untreated group (both $P \leq 0.03$; Fisher exact test).

monitors or in the houses. All colonies infesting structures were *R. flavipes.* Three of the eight structures were each infested by two colonies, whereas the other five structures were attacked by lone colonies.

Effect of Fipronil on Colony Survival. The numbers of treated, likely treated, and untreated colonies present on the day of treatment and 90 d after treatment are shown in Table 2. Also shown are the numbers of colonies in each category detected again at least once between 90-d posttreatment and 3 yr after treatment. None of the 11 treated colonies was found on or after the 90-d posttreatment sampling date and were presumed to be eliminated. Few of the 12 likely treated colonies were found during this period, although three (25%) were found repeatedly up to the end of the study. In contrast, the untreated colonies were much more likely to be redetected, with 60% of the colonies found again after the 90-d sampling period. The difference in the redetection rate among treatments for the period between 90 d posttreatment and the end of the study was highly significant ($P < 0.0001$; Fisher exact test) but not at the 90-d sampling date ($P > 0.75$; Fisher exact test). The difference among treatments was due to the high detection frequency of the untreated colonies compared with the other two groups ($P < 0.001$ and $P < 0.03$ for treated and likely treated colonies, respectively; Fisher exact test), whereas there was no significant difference between the treated and likely treated colonies during this period ($P > 0.10$).

The elimination of the treated and likely treated colonies was accompanied by a cessation of termite activity in or near the house as evidenced by the disappearance of colonies in the houses and the inner ring of monitors after the 90-d monitoring period, although termites continued to be present in the outer ring of monitors (Figs. 1–4 and) and in the natural areas throughout the study (Figs. 1 and 2).

Of the 11 treated colonies, all of which were attacking structures, four had somewhat extended foraging ranges with foragers occupying monitoring stations in the outer ring and/or natural areas at the time of treatment (e.g., Fig. 2). The maximal distance from the house and therefore the treatment zone in these four colonies ranged from 6 to 13.5 m. As noted, all active workers from these colonies disappeared by 90 d after treatment, indicating that at least in some cases, field applications of fipronil can affect foragers located >10 m from the treatment zone. The largest of the infesting colonies had an estimated foraging area of 365 m^2.

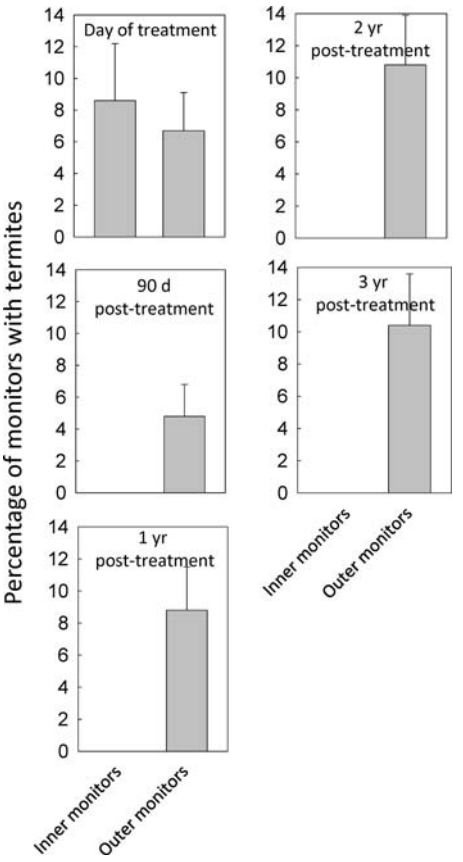

Fig. 3. Subterranean termite activity at monitors (mean percent ± SE) located within 0.5 m from the foundation wall (inner monitors) and those further from the structures (2–20 m from the foundation wall; outer monitors) before treatment and at four time points after treatment.

Discussion

Our findings show that fipronil treatment can have severe impacts on subterranean termite colonies in the field, with strong colony suppression probably resulting in colony elimination. In this study, we used molecular markers to genetically "fingerprint" colonies so that their fate could be tracked after termiticide application. All 11 of the treated colonies—the colonies known to be infesting structures and were targeted by termiticide application—went undetected within 90 d of treatment and were not detected again during the 3-yr study period. Given the intense sampling effort we undertook to find any workers of these colonies, including 11 or more inspections of all monitoring stations after the 90-d posttreatment date, the most likely explanation for the continued absence of these colonies was that they were eliminated by the treatment. This is in contrast to 60% of the untreated colonies that were detected again during the same

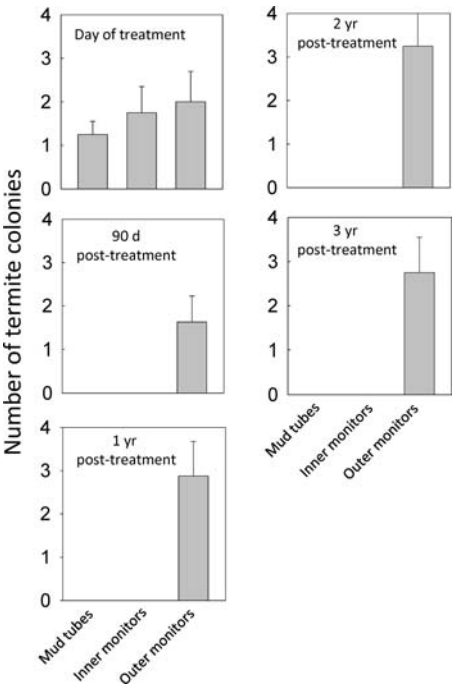

Fig. 4. Mean number (±SE) of subterranean termite colonies found in mud tubes on the structure, in monitors located within 0.5 m from the foundation wall (inner monitors), and in monitors located further from the structures (2–20 m from the foundation wall; outer monitors) before treatment and at four time points after treatment.

period, and many of these were found on multiple occasions. The likely treated colonies—those occupying monitoring stations within 0.5 m of the foundation where the termiticide was applied and were therefore probably exposed to the treatment—showed an intermediate response, with only 25% (three of 12) redetected during the study.

Our approach of using intensive sampling and genetic fingerprinting is a powerful means to track individual colonies, but like any method to determine the fate of colonies under field conditions, it has certain limitations in definitively proving colony elimination. As we noted in our previous studies of colony level effects of baiting (Vargo 2003) and liquid application of imidacloprid (Parman and Vargo 2010), our ability to detect the continuing presence of a colony depends on collecting samples of individuals from that colony in wood debris and monitors. Thus, it is possible that some colonies survived the treatment and continued to persist on the property but we failed to find them again after the pretreatment sampling period because they were not encountered again in either the monitors or wood debris. It is also possible that some of these colonies migrated out of the study site and persisted outside of the monitored area. The untreated colonies thus serve as important controls for our ability to attribute apparent colony-level effects to the fipronil treatment, because such colonies provide the baseline for the likelihood of not redetecting colonies due to reasons other than elimination by insecticide treatment, including a failure of the sampling method to detect colonies that are present, relocation of colonies out of the sampled area, or death from natural causes. It is telling that although we redetected most (60%) of the untreated colonies at least once after 90 d posttreatment, we failed to detect a single treated colony during this time. In our view, the most likely explanation for our failure to redetect any treated colonies during the intensive 3-yr monitoring period is that they were eliminated, although we cannot exclude the possibility that some of the treated colonies persisted after treatment with greatly reduced population sizes and were not encountered again.

The strong colony-level effects and the likely elimination of colonies after fipronil treatment could be due to two possible nonexclusive mechanisms. First, foragers contacting treated soil may transfer sufficient amounts of fipronil throughout the colony to kill all members. Several studies have shown that fipronil is transferred from exposed workers to naïve subterranean termites in the laboratory in *Reticulitermes* spp. (Mulrooney et al. 2007, Saran and Rust 2007, Spomer et al. 2008, Bagnères et al. 2009) and in *Coptotermes* spp. (Ibrahim et al. 2003, Shelton and Grace 2003, Tsunoda 2006). Results concerning the strength of transfer and effects on recipients vary depending on the route of exposure of donor termites, temperature (Spomer et al. 2008), the ratio of recipients to donor (Spomer et al. 2008), and the castes involved in transfer (Ibrahim et al. 2003). Working with *R. flavipes*, Bagnères et al. (2009) reported that nearly half of the radiolabeled active ingredient was transferred from exposed to naive termites with a 1:1 ratio of donors to recipients, regardless of whether the donors were exposed through contact with treated soil or feeding. These authors also reported high mortality of recipient individuals. Saran and Rust (2007) performed a series of horizontal transfers in *R. hesperus* and found significant mortality in the initial recipients, but these recipients were unable to pass on lethal amounts of fipronil in a subsequent round of transfer. These authors found that most of the transfer occurred through contact and not trophallaxis. Saran and Rust (2007) also observed that termites exposed to lethal amounts of fipronil do not travel >1.5 m from the treated zone, leading them to conclude that horizontal transfer was not a major cause of the efficacy of fipronil treatments in the field. Su (2005) reported similar findings in a laboratory study of *C. formosanus* and suggested that fipronil would not have lethal effects beyond a distance of 5 m within a colony of this species.

A second potential mechanism by which fipronil may exert colony-level effects is direct contact of treated soil by most or all of a colony's foragers leading to eventual colony death over time due to attrition. Evidence against this possible mechanism, at least in *C. formosanus*, comes from the results of the abovementioned study by Su (2005) performed in extended foraging arenas in the laboratory. Nearly all mortality due to exposure to fipronil treated soil in this study

occurred ≤5 m from the treatment zone and foragers tended to avoid areas with corpses, eventually repelling them from the treated soil. Su (2005) reported that only 25–35% of the foragers were killed by the treatment and suggested that portions of the colony located >5 m away from the treated area would be not be strongly affected, limiting colony wide impact on this species. These conclusions are consistent with our findings. Colonies of *R. flavipes* in our study area tend to be localized with foraging areas generally not extending beyond five linear meters (Parman and Vargo 2008), although one of the treated colonies (property HI, Fig. 2) extended beyond ≈13.5 m from the treatment zone and was eliminated, suggesting that at least in *R. flavipes*, the colony-level effects of fipronil treatment can occur across distances of ≥10 m. From our results, it is clear that whatever the mechanism responsible, fipronil treatment severely impacted field colonies of *R. flavipes*, probably resulting in their elimination.

One of the striking findings of the current study was the complete cessation of activity in the inner monitors after treatment. These results suggest that the area immediately around the structure remained largely or completely termite free within 90 d of treatment. These results are consistent with those of Potter and Hillery (2002) who found a lack of *R. flavipes* activity within several meters of treated structures, and those of Ripa et al. (2007) who found a decline in active monitors within 2 m of the treatment zone in *R. flavipes*. In a study very similar to the present investigation but involving imidacloprid, we also found decreased activity of the inner monitors, although activity remained in some of those monitors throughout the 2-yr duration of the previous study (Parman and Vargo 2010).

We found that some 25% of the likely treated colonies survived and persisted throughout the study period. The reasons for the survival of these colonies are not clear. Because these colonies were occupying monitors within 0.3 m of the foundation wall, it seems likely that they would have been exposed to the treatment applied close to the foundation wall by trenching and rodding. It could be that although many workers in most of these likely treated colonies contacted the nearby treated soil, few or no workers in other likely treated colonies entered the treatment zone despite being active very close to it, thereby limiting the effects of the active ingredient on some colonies. Nonetheless, there seemed to be a strong effect on most of these colonies. This stands in contrast to our results with imidacloprid (Parman and Vargo 2010), in which 67% of the likely treated colonies survived after 2 yr, a survival rate very similar to the untreated colonies in that study (71%).

The redetection rate of 60% of the untreated colonies in the current study was slightly lower than the 71% found in an earlier similar study we conducted using the same methods and in the same geographic region to evaluate the colony-level effects of imidacloprid (Parman and Vargo 2010). It is not clear why there was a slightly lower rate of detection of untreated colonies in the current study, especially because it was conducted over a period of 3 yr, giving more opportunity to encounter any colonies present than in the previous study that lasted only 2 yr. One possibility is that there were differences between the individual properties used in the two studies such that untreated colonies in the current study were more difficult to detect, possibly because 1) they disappeared more frequently due to greater natural mortality, 2) more of them moved out of the study area, or 3.) they were less likely to feed on monitors because there were more natural food sources present on the properties. Another possibility is that the effects of fipronil treatment were stronger than those of imidacloprid so that some of the untreated colonies eventually entered treated soil during the course of the study and suffered colony-level effects. This possibility is supported by the higher across the board effects of fipronil on treated and likely treated colonies (100 and 75% disappearance, respectively, compared with 75 and 23% with imidacloprid), suggesting that the fipronil treatment in the current study affected all classes of colonies to a greater extent than did imidacloprid in our previous study (Parman and Vargo 2010).

In discussing the effect of nonrepellent liquid termiticides in a review of new technologies for termite control, Su (2002) rightly pointed out nearly a decade ago that there were that "no published data to date to demonstrate their impact on field populations of subterranean termites." The current study together with our previous study on imidacloprid (Parman and Vargo 2010), providing data on 20 properties treated with nonrepellent liquid termiticides, is starting to fill that gap. In both cases, there was a clear effect of insecticide treatment on colonies; all of the structures were termite free within 90 d, and 20 of the 23 treated colonies disappeared from the study areas altogether, probably because they were eliminated. In addition, termite activity close to the foundation walls dramatically decreased in the case of imidacloprid and ceased altogether in the case of fipronil. In these respects, these two nonrepellent liquid termiticides have effects much like baits. In a previous study performed in Raleigh, NC, using similar methods to those applied here, we (Vargo 2003) found evidence for colony elimination and reduced termite activity in an apartment complex treated with baits containing hexaflumuron. In that study, colonies visiting >230 in-ground monitoring stations located near the foundation wall were baited and tracked for a 3-yr period. All of the colonies tracked for at least 1 yr after baiting—34 *R. flavipes* colonies and one *R. virginicus* colony—disappeared from the study and were presumed to be eliminated. In that study, activity at the monitors decreased by 67% over 2 yr. In our previous study on colony-level effects of imidacloprid treatment, we also saw about a 67% decrease in the activity of the inner monitors (Parman and Vargo 2010). However, in the current study, we saw an even more pronounced effect with complete cessation of all activity in the inner monitors over a 3-yr period after fipronil treatment.

Using molecular markers to track colonies in the field with both fipronil in the current study and imidacloprid in our previous study (Parman and Vargo 2010), we have shown that at least these two nonrepellent liquid termiticides can have strong colony-level effects on subterranean termites very similar to the effects of baits, severely suppressing colony populations and in many cases resulting in their elimination.

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