Colony-Level Effects of Imidacloprid in Subterranean Termites (Isoptera: Rhinotermitidae)

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J. Econ. Entomol. 103(3): 791–798 (2010); DOI: 10.1603/EC09386

ABSTRACT We determined the impact of imidacloprid (Premise) on colonies of *Reticulitermes* spp. (Isoptera: Rhinotermitidae) through soil applications in the field. We selected 11 houses in the Raleigh, NC, area with active termite infestations. In-ground monitoring stations (mean = 75.9 stations) were installed around each house, and samples of termites visiting the monitors, in mud tubes, as well as samples from wood debris in the yard, were collected monthly for up to 14 mo to determine the numbers and locations of colonies present before treatment. We used microsatellite genetic markers to identify individual colonies present on each property. All houses were treated with Premise 75 WSP by using an exterior perimeter/interior spot treatment. After treatment, termite samples were collected monthly for 3 mo and then quarterly for 2 yr to track the fate of colonies. Of the 12 treated colonies (those attacking structures), 75% disappeared within 90 d and were not detected again. In contrast, only 25% of 48 untreated colonies (located 2 m or further from the treatment zone) and 40% of the six likely treated colonies (located within 0.5 m of the treatment zone but not known to be attacking the structure) were not detected again during the study. Our findings are consistent with strong colony-level effects of soil treatments with imidacloprid, resulting in the suppression or elimination of *Reticulitermes* spp. colonies in many cases.

KEY WORDS *Reticulitermes flavipes*, *Reticulitermes virginicus*, colony suppression, colony elimination, microsatellites

Subterranean termites are major economic pests throughout much of the United States, where costs from control and damage may total as much as $11 billion annually (Su 2002). Remedial and preventative treatments for subterranean termites consist mainly of liquid termiticides applied to the soil, or to a lesser extent baiting systems (Anonymous 2002). Over the last decade or so, relatively slow acting, nonrepellent liquid termiticides, especially fipronil (Termidor, BASF Corp., Research Triangle Park, NC) and imidacloprid (Premise, Bayer Environmental Sciences, Research Triangle Park, NC), have largely replaced pyrethroids and organophosphate insecticides as the active ingredients of choice in termite soil treatments (Anonymous 2002).

Imidacloprid belongs to the neonicotinoid class of insecticides (Matsuda et al. 2001). It exhibits delayed mortality against termites (Ramakrishnan et al. 2000, Gahlhoff and Koehler 2001, Thorne and Breisch 2001, Haagsma and Rust 2007), and it can be transferred from exposed termites to naïve termites within a colony (Haagsma and Rust 2007), suggesting that it may have activity on termite colonies extending beyond the immediate zone of treated soil (Thorne and Breisch 2001). Reports from field studies give a mixed picture of the potential colony-level effects of imidacloprid treatment. Osbrink and Lax (2003) reported observing symptomatic termites of *Coptotermes formosanus* (Shiraki) 46 m from the site of imidacloprid application, suggesting that toxic doses can be transferred over long distances. However, in a separate study, Osbrink et al. (2005) found that monitors containing *C. formosanus* located 1–3 m from treated buildings did not experience reduced activity, although it was not determined by these authors whether the monitored colonies were actually exposed to the treated soil. Potter and Hillery (2002) reported that treatment of structures infested with *Reticulitermes flavipes* (Kollar) colonies affected termites in some but not all monitoring stations located 0.3–3.0 m from treated areas. However, this study did not employ methods to track individual colonies over time, so that it was not determined whether termites found in monitors several months after insecticide application belonged to the same colonies as those receiving the initial treatment.

More definitive determination of colony-level effects of termiticides requires that colonies known to be exposed to insecticide be identified and followed over time to determine their fate. Molecular genetic markers provide powerful tools for identifying indi-

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Table 1. Characteristics of study properties and pretreatment sampling period

Property	Construction type	Length of pretreatment monitoring period (d)	No. monitors	No. times sampled	No. samples collected	No. colonies in house	Total no. colonies detected on property	Total no. colonies per ha
BU	Crawl space	265	82	10	29	1	7	43.2
CH	Crawl space	231	62	7	21	2	2	29.1
LC	Crawl space	296	58	6	17	1	6	123.6
PE	Crawl space	296	114	8	55	1	9	23.9
RI	Mixed, crawl space, basement	318	79	7	17	1	5	28.7
RB	Crawl space	160	82	5	41	1	5	17.9
SO	Basement	415	89	12	105	1	4	89.9
SA	Floating slab	216	72	4	33	1	6	74.1
TH	Crawl space	244	60	5	20	1	6	67.4
WM	Crawl space	276	59	5	18	1	6	87.2
WP	Crawl space	431	78	13	65	1	15	185.3
Mean ± SD		286.2 ± 80.6	75.9 ± 16.7	7.5 ± 3.0	38.3 ± 27.4	1.1 ± 0.3	6.5 ± 3.3	70.0 ± 50.8

vidual colonies and tracking them over time (Vargo and Husseneder 2009). Microsatellite markers have been used to successfully identify and track colonies after bait treatment over periods of 3–4 yr in *Reticulitermes* spp. (Vargo 2003, Thoms et al. 2009) and in *C. formosanus* (Aluko and Husseneder 2007).

The objective of this study was to determine whether colonies of subterranean termites that were infesting houses would be eliminated by applications of imidacloprid (Premise). To accomplish this aim, we conducted a study on residential properties located in the Raleigh, NC area with active structural infestations of *Reticulitermes* spp. We first identified the colonies attacking the structures as well as other colonies located on the property and then treated the houses using an exterior perimeter/interior spot treatment application. We tracked the fate of colonies over a 2-yr period. Our results suggest that field applications of imidacloprid have strong colony-level effects on *Reticulitermes* spp., leading to colony suppression and possible colony elimination in most cases.

Materials and Methods

Study Houses. Eleven residential structures in and around Raleigh, Wake County, NC, with active subterranean termite infestations were selected for this study. Of these structures, 10 were stand alone single family dwellings and one was part of a four unit building. These houses are a subset of the 20 houses reported on by Parman and Vargo (2008) as part of a study of species abundance and population density of subterranean termite colonies on residential properties in central North Carolina. The 11 houses in the current study averaged 634 m^2 (2,578 $feet^2$) ± SD 58.9 m2 (634 $feet^2$), ranging from 176 to 376 m^2 (1,830–4,051 $feet^2$). Construction type varied somewhat (Table 1): eight houses had crawl spaces, one house had a basement, one house had a mixed basement/crawl space, and one house had a floating slab.

Monitoring stations were installed around the structures and in the yard areas between 10 April 2002 and 15 October 2003. For each property, we installed the monitors either in a single day or over two consecutive days. We constructed monitoring stations from 6-cm-diameter polyvinyl chloride (PVC) pipe cut to 30-cm lengths with two grooves cut halfway up the tubes. Inside the tubes, we placed two pieces of 20-cm-long strips of pine that were in direct contact with the soil. The stations were inserted into prebored holes and covered at ground level with a PVC cap. On average, we installed 75.9 ± SD of 16.7 (range, 58–114) monitors per residence. We placed the monitors in concentric rings around the house (Figs. 1 and 2). The inner ring was located ≈0.5 m from the foundation wall and placed 2–6 m apart. We installed an outer ring of monitors ≈6 m away from the first ring of monitors, and occasionally, we also installed a third ring of monitors ≈6 m beyond the second ring. In one case (property WP), wooden survey stakes were placed as accessory monitors under a deck and inside the crawl space.

Pretreatment Termite Sampling. Monitors were checked monthly for the presence of termites a mean of 7.5 ± 3.0 times over a period of 9.5 ± 2.7 mo (range, 5.3–14.4) before treatment. Mud tubes and any infested wood in the structure were also inspected for termites. "Natural areas," i.e., other areas in the yard containing wood debris, stumps, wood piles, and so on, also were checked at least twice before treatment. Termite workers and, when present, soldiers were collected from mud tubes, monitoring stations, and wood debris, put into vials containing 95% ethanol and stored at −20°C until DNA extraction. On average, 38.3 ± 27.4 pretreatment samples were collected per property. A final sample was collected either the day of treatment or the day before treatment. As described below, we genotyped the pretreatment samples to determine colony affiliations, and we then constructed maps showing the locations of all active colonies detected on the properties (e.g., Figs. 1 and 2).

Treatment. Termiticide was applied by a commercial licensed pest management professional using Premise 75WSP at the label rate of 0.05%. We applied an exterior perimeter/interior spot treatment according to current label instructions. The exterior was treated with the standard rod and trench method around foundation walls, including drilling of slabs,

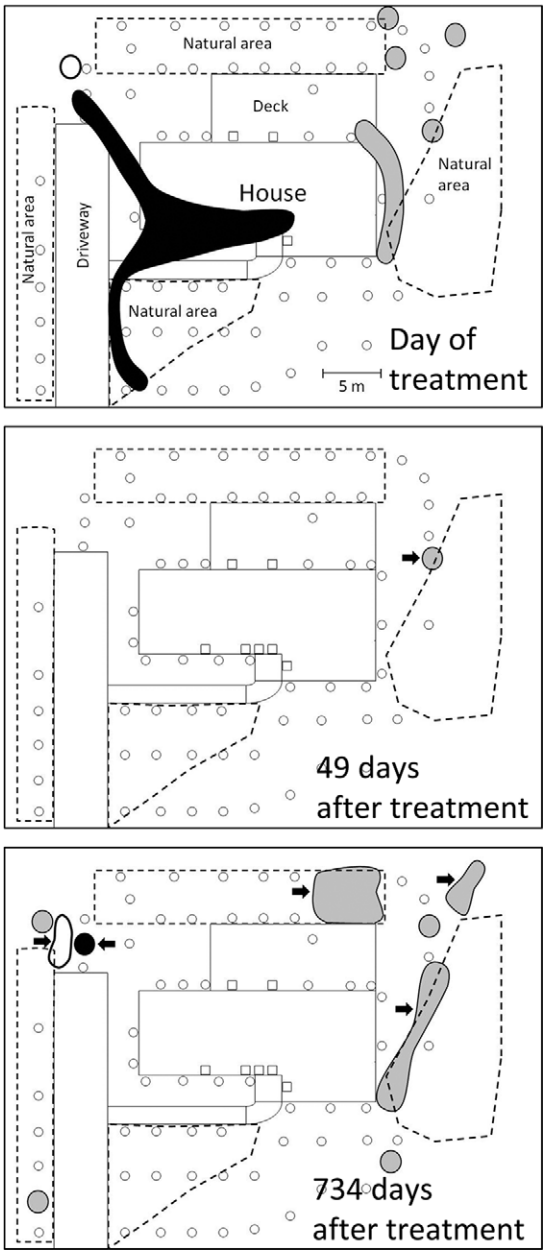

Fig. 1. Locations and observed foraging areas of subterranean termite colonies around property BU before treatment and at two time points after treatment. Small open circles represent in-ground monitoring stations; small open squares represent wooden stake monitors. The *R. flavipes* colony infesting the structure is shown in black. Arrows indicate colonies that were present before treatment and were found again at the latter time points. *R. flavipes* colonies are shown in black and gray; *R. virginicus* are shown in white.

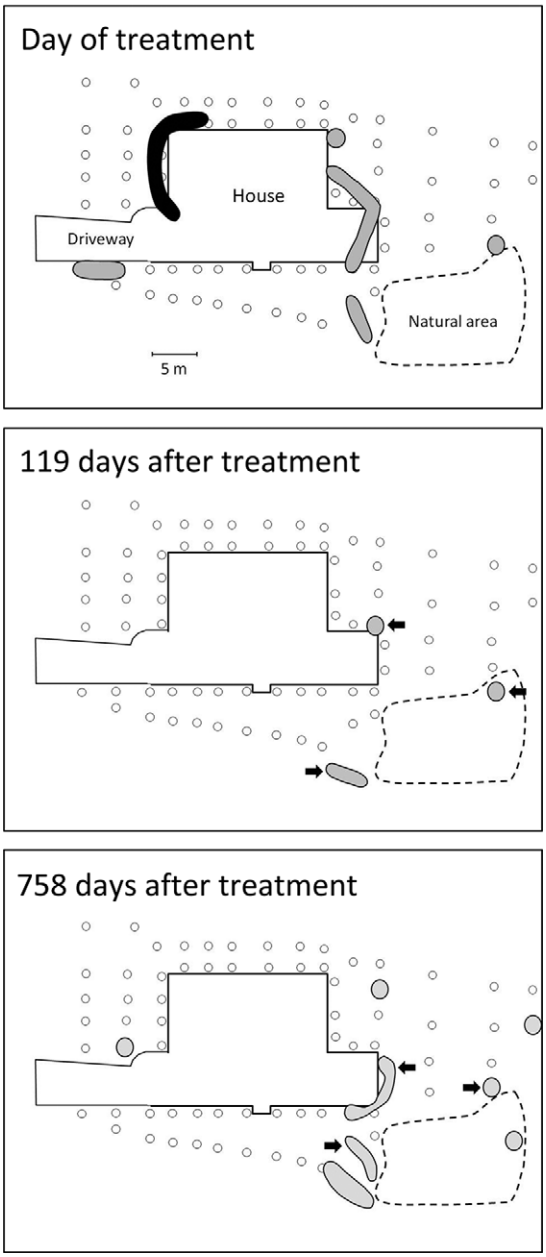

Fig. 2. Locations and observed foraging areas of subterranean termite colonies around property SA before treatment and at two time points after treatment. Small open circles represent in-ground monitoring stations. The colony infesting the structure is shown in black. Gray denotes other colonies found on the property. Arrows indicate colonies that were present before treatment and were found again at the latter time points. All colonies at this property were determined to be *R. flavipes*.

walkways and porches where appropriate. The interior areas around active mud tubes or other signs of termite activity were treated as specified on the label by soil treatment and, where appropriate, drilling hollow blocks.

Posttreatment Termite Sampling. Each property was monitored for at least 2 yr after treatment according to the following schedule. After treatment, monitors and mud tubes were checked for the presence of living termites weekly for 4 wk, then monthly for 6 mo,

after which time they were checked quarterly for an additional 1.5 yr. We followed this schedule as closely as possible, but in some cases the sample schedule was shifted by a month or so. The sample collected closest to the 90-d time period (range, 49–105 d) was taken to represent this date. Natural areas were sampled semiannually for the duration of the study. Samples of termites present were collected and held in vials containing 95% ethanol at −20°C until DNA extraction.

As a measure of overall termite activity over the course of the study, the number of monitors with active termites present was totaled for each property at the following time points: day of treatment (assessed before the treatment was applied), ≈90 d after treatment and ≈730 d (2 yr) after treatment. Termite presence at these time points provided a snap shot of termite activity before treatment, 90 d posttreatment, at which time all structures were free of termite infestations, and at the end of the 2-yr study period.

DNA Analysis for Termite Colony Affiliation. Genomic DNA was extracted from whole termite bodies using either the DNeasy kit (QIAGEN, Valencia, CA) or a modification of the Gentra PureGene kit (Gentra Systems, Inc., Minneapolis, MN). We identified samples to species using the polymerase chain reaction (PCR)-restriction fragment length polymorphism method of Szalanski et al. (2003) on a portion of the cytochrome oxidase II (COII) gene. The pretreatment samples were genotyped at 10 microsatellite loci: *Rf 24-2*, *Rf 21-1*, *Rf 15-2*, *Rf 6-1*, *Rf 5-10*, and *Rf 1-3* (Vargo 2000), and *Rs 16*, *Rs 33*, *Rs 62*, and *Rs 76* (Dronnet et al. 2004). We followed the PCR conditions described by Vargo (2000) and Dronnet et al. (2004). We ran the resulting fluorescently labeled products on 6.5% polyacrylamide gels using a Li-Cor 4300 automated DNA sequencer (LI-COR Biosciences, Lincoln, NE). Size standards were run about every tenth lane, and the size of the detected microsatellite bands was determined using GeneProfiler version 3.56 (Scanalytics, Inc., Fairfax, VA).

To determine colony affiliation of collected foragers, we analyzed termites from each station in which they were present. In the pretreatment samples, we did not always analyze individuals collected from the same station on different dates. For a given monitoring station, we analyzed foragers collected over at least three time points, including the first and last samples collected. In all cases, the same colonies were found to be present during these three time points, and we therefore conlcuded that all termites found in the same station belonged to the same colony during the entire pretreatment sampling period. All samples taken from mud tubes and natural areas during the pretreatment period were analyzed.

All samples collected in the 3-mo period after treatment were analyzed to determine colony identity. After this period, only a subset of samples was analyzed from monitoring stations in which termites were frequently found. In such cases, we again analyzed samples from three time points, including the first and last samples taken as well as one time point in between. There were eight cases in which there was a change in the identity of a colony visiting a particular monitor. In four of the cases, the treated colony (see Effect of Imidacloprid on Termite Colonies below for a description of the different classes of colonies) was replaced by another colony. All of the natural area samples collected for the entire duration of the study were analyzed.

Table 2. Number of alleles (N_A) and observed heterozygosities (H_O) present in the loci used to determine colony affiliation in posttreatment samples

Species	*Rf 24-2*		*Rf 15-2*		*Rf 21-1*	
	N_A	H_O	N_A	H_O	N_A	H_O
R. flavipes $N = 167$	36	0.92	4	0.27	45	0.86
R. hageni $N = 12$	12	0.58	6	0.37	15	0.68
R. virginicus $N = 5$	3	0.62	1	0.00	10	0.63

N is the number of colonies sampled as previously reported by Parman and Vargo (2008).

Colony identity was determined by comparing the genotypes of groups of foragers by means of an exact test of genotypic differentiation using GenePop on the Web (Raymond and Rousset 1995). Samples collected from the same property were considered to belong to the same colony if they shared the same genotypes and if they did not differ significantly ($P > 0.05$) in genotype frequencies. For the pretreatment samples, colony assignments were based on the genotypes of at least 10 individuals per sample at all 10 microsatellite loci. These samples were included as part of a larger study on colony breeding structure (Parman and Vargo 2008). For the post treatment samples, we genotyped at least five individuals per sample at *Rf 24-2* and *Rf 15-2*, and some samples were also genotyped at *Rf 21-1*. Given the large number of alleles and high heterozygosity of *Rf 24-2* in the study population (Table 2), especially for *R. flavipes*, which composed >90% of all colonies in the study population, this locus alone was usually sufficient to distinguish colonies. In some cases, *Rf 21-1*, also a highly variable locus, provided confirmatory information on colony identity. Although marker variability was lower in *R. hageni* and *R. virginicus*, mostly due to the small number of colonies sampled, colony assignments for these species were straightforward because there were very few colonies of each, usually only a single colony, present on any given property.

Effect of Imidacloprid on Colonies Survival. Colonies identified on each property before treatment were placed into one of three groups to distinguish among treatment levels. Colonies that were known to be infesting a structure, i.e., present in shelter tubes along the foundation wall or present in structural elements, were the targets of the treatment. These were labeled treated colonies. Colonies that were occupying any of the monitoring stations located in the inner ring were labeled as likely treated, because the treatment was applied to the entire exterior foundation wall and it seemed reasonable to assume that colonies foraging in monitors located only 0.3–0.6 m from the treated soil would have

been exposed to the treatment. Colonies located exclusively in the outer ring of monitors or in natural areas were considered untreated colonies.

For each property, we determined the number of colonies in each of the three categories (treated, likely treated, and untreated) that were either found again or not found again ≈90 d after treatment and anytime between 90-d posttreatment and the end of the study, ≈2 yr (730 d) after treatment. We used 90 d for the first time point because all activity of termites infesting structures had ceased by this time. We reasoned that any colony-level effects of the treatment should therefore be evident at 90 d posttreatment. Both the monitoring stations and natural areas were sampled at the 90-d time point. Between 90 d posttreatment and the end of the experiment, monitors were checked at least six times and natural areas were checked at least three times. If a sample collected during this time period was assigned to a colony present during the pretreatment period, then this colony was considered to have persisted. Colonies present during the pretreatment period but not detected again after treatment were considered to have disappeared. Not all colonies present in the pretreatment period were found again later in the study, including those in natural areas which were presumably untreated. To test the hypothesis that the treatment resulted in the elimination of treated colonies but not untreated colonies, we performed a Fisher exact test on the numbers of colonies in each of the three categories—treated, likely treated, and untreated—that were either found again or not found again. This was performed for both the 90 d posttreatment sample and the 90- to 730-d posttreatment period. At the end of the study, all houses were inspected by a pest management professional for signs of termite infestation.

Colony Foraging Area Estimation. The foraging area occupied by a colony was estimated by calculating the area enclosed by the simplest polygon connecting all the sampling points containing workers from that colony as described in Parman and Vargo (2008). Colonies present at only a single sampling site were assigned an area of 1 m^2, and those present at only two points were assigned an area equal to the distance between the points in square meters, e.g., a colony occupying two stations 5 m apart was assigned a foraging area of 5 m^2.

Results

General Description of Colonies Present Before Treatment. Details of the construction type for each property as well as pretreatment characteristics, numbers of monitors, numbers of samples collected and numbers of colonies attacking each structure are given in Table 1. All but one of the properties had a single colony infesting the structure at the time of treatment; property CH had two colonies simultaneously infesting the house through a series of separate mud tubes. Of the 12 colonies infesting structures, 11 were *R. flavipes* and one was *R. virginicus.* During the pretreatment period, we found a total of 72 colonies present on all properties, including colonies attacking the structure and those in monitors and natural areas in the yard, for an average of 6.5 ± 3.3 colonies per property. This translated into an average density of 70.0 ± 50.8 colonies per ha (28.3 ± 20.6 colonies per acre) for the 11 properties.

Table 3. Effect of imidacloprid treatment on colonies of *R. flavipes* on residential properties in the Raleigh, NC, area

Colony type	Pretreatment count	Count 90 d posttreatment		Count 730 d posttreatment	
		Detected	Not detected	Detected	Not detected
Treated	12	2	10	3	9
Likely treated	9	4	5	6	3
Untreated	49	20	29	35	14

Effect of Imidacloprid on Colony Survival. Figures 1 and 2 show two representative properties with the locations of colonies present at the time of treatment 90 d posttreatment (or the sampling time closest to this time), and 730 d posttreatment. The colony infesting the structure in the BU property continued to persist throughout the study, whereas that in the SA property was not found at the 90-d sample or at any point later on. Table 3 shows the numbers of colonies of each type found on all properties at the time of treatment, at 90 d posttreatment and 90 to 730 d posttreatment. We were unable to find workers belonging to 10 of the 12 (83%) treated colonies 90 d after treatment. The frequency of treated colonies present at 90 d (17%) was less than half of the likely treated (44%) and untreated (41%) colonies. However, there was no significant difference among treatments at the 90 d posttreatment mark ($P = 0.31$; Fisher exact test). During the longer monitoring period from 90 to 730 d, we found additional colonies of all three categories, but mainly in the likely treated and untreated classes, where we were able to detect 67 and 71% of the original colonies, respectively, compared with only 25% (three of 12) of the treated colonies. The treatments differed significantly in the frequencies with which they were redetected ($P = 0.01$; Fisher exact test). To determine whether the significance value obtained was largely due to one of the colony classes, we subdivided the contingency table and performed pairwise tests using 2 by 2 tables. The results revealed that the difference was mainly due to the frequency of the treated colonies, because removal of this category produced a non significant table ($P = 0.53$), whereas removal of the likely treated group produced a highly significant table ($P = 0.004$) and removal of the untreated colonies produced a table close to significance ($P = 0.063$). From this we concluded that the treatment resulted in a significantly lower frequency of treated colonies being redetected compared with untreated colonies, most likely because most of the Treated colonies had been eliminated. The likely treated colonies appeared to experience an intermediate effect, because their frequencies did not differ significantly from the other two categories.

The activity of monitors located near the structure (inner monitors) and those further away from the foundation wall (outer monitors) is shown in Fig. 3. There was a decrease in termite activity in all

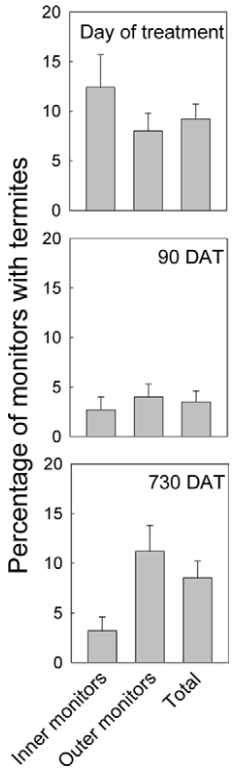

Fig. 3. Subterranean termite activity at monitors located within 0.5 m from the foundation wall (inner monitors) and those further from the structures (2–20 m from the foundation wall; outer monitors) throughout the study period. Three time points are shown: day of treatment, 90 d after treatment, and 730 d after treatment.

monitors 90 d after treatment. However, by the 2-yr mark, activity had rebounded in the outer monitors, but not in the inner monitors where fewer than 5% had termite activity. In addition to the 72 colonies we detected during the pretreatment period, we identified an additional 59 "new" colonies after the time of treatment. Most of these (55%) were located in natural areas; the remainder of colonies was found in the outer monitors in the yard.

Colony Foraging Areas. The *R. flavipes* colonies infesting the houses were more expansive than other colonies located elsewhere on the properties (Table 4). At the time of treatment, colonies infesting structures had on average 3 times the linear foraging distance ($t_{25.9} = 3.185$, $P < 0.005$, two tailed test assuming unequal variances) and 4 times the foraging area as the other colonies present, although this latter difference was not significant ($t_{14.0} = 2.06$, $P = 0.058$, two tailed test assuming unequal variances). Of the three treated colonies still present at the end of the study, two were *R. flavipes* and one was *R. virginicus*. All of the colonies seemed to have undergone a reduction in the spatial area they occupied. By the end of the study, the two *R. flavipes* colonies went from foraging areas of 109.5 and 56.3 m^2 before treatment to one and 11.5 m^2, respectively. The *R. virginicus* colony, which was the most expansive colony in the study, went from 318.2 m^2 before treatment to 36.1 m^2 by the end of the study. Although the surviving treated colonies occupied larger initial foraging areas on average than did the treated colonies that were not found again (59.3 ± 48.8 and 15.4 ± 11.89 m^2, respectively), this difference was not significant ($P > 0.25$; *t*-test).

Table 4. Estimated size of foraging areas (mean ± SD) of *R. flavipes* colonies infesting houses and other colonies located on the same properties

	n	Linear distance (m)	Foraging area (m^2)
Infesting houses	11	12.0 ± 6.5	27.7 ± 31.6
Not infesting houses	51	3.9 ± 4.5	6.3 ± 16.0

Discussion

These results provide strong evidence that field applications of imidacloprid can have strong effects on subterranean termite colonies. Despite intensive sampling for 2 yr posttreatment, nine of 12 *Reticulitermes* spp. colonies that were treated with Premise were not detected again 90 d or longer after treatment, compared with 29% of untreated colonies that went undetected during the same time period. The most likely reason for the lower redetection frequency of the treated colonies was due to the elimination of many of them by imidacloprid application. Admittedly, we cannot know with certainty that treated colonies that went undetected again were eliminated; we can only state that we did not find evidence for them in the monitors or wood debris in the yard. It is possible that they were present but did not visit any of the monitors because their forager populations were reduced to small numbers of individuals who were not encountered by our sampling methods. In this regard, the untreated colonies served as controls because they provided baseline data for the frequency of redetection of colonies not exposed to termiticide. Over the course of the study, we were able to redetect 71% of the untreated colonies that were identified before the treatment was applied. The untreated colonies that were not detected again were missed by our sampling methods, moved away from the study site or died of natural causes. Using 71% as a baseline redetection frequency for colonies present on a property in the absence of termiticide application, we would expect to redetect eight to nine of the 12 treated colonies under the null hypothesis that the treatment had no effect on colonies. Instead, we redetected only three colonies, a significantly smaller number than expected, allowing us to reject the null hypothesis. Applying the 71% redetection rate to the treated colonies that disappeared from the study, we can conservatively estimate that of the nine treated colonies that were not redetected, six ($9 \times 0.71 = 6.39$) were either eliminated or had their forager population severely reduced as a result of the treatment, whereas three colonies may have been present but went undetected. Thus, at least

half of the 12 treated colonies in this study experienced severe colony level effects, possibly resulting in colony death. In all cases, detectable termite activity in the houses ceased within 90 d of treatment and did not return for the duration of the 730 d study.

It is clear from our results that there was a strong colony-level impact on most treated colonies. Potter and Hillery (2002) reported similar findings in a field study conducted in Kentucky. These authors installed a limited number of in-ground stations around four infested structures to which exterior treatments of Premise were applied. Each property was infested by a single colony of *R. flavipes*, as determined by marking termites present in some stations with a lipid-soluble dye and noting the presence of dyed termites in other stations. The stations, located 0.3–3.0 m from the site of treatment, were monitored for 13–14 mo after treatment. During this time, activity ceased in 19 of 33 stations, including all six stations at one site, and Potter and Hillery (2002) reported seeing dead termites in several of the stations.

There are two potential nonexclusive mechanisms by which imidacloprid applied to the soil could exert colony-level effects. First, there could be sufficient horizontal transfer of imidacloprid from exposed foragers who contact treated soil to naïve nestmates away from the treatment zone affecting termites throughout the entire colony. However, a recent laboratory study of *Reticulitermes hesperus* Banks by Haagsma and Rust (2007) found that ^{14}C-labeled imidacloprid was not efficiently transferred among colony members away from the area of treatment, calling into question the importance of horizontal transfer within colonies. These authors reported that transfer, when it occurred, involved contact only, and occurred at rates of 7.5–16.5%. A second potential mechanism that could affect large numbers of individuals in colonies is direct contact of treated soil by all or most individuals in the colony and eventual colony attrition over time. *Reticulitermes* spp. workers exposed to lethal or sublethal doses of imidacloprid rapidly become immobilized and most do not move out of the treated zone (Thorne and Breisch 2001, Haagsma and Rust 2007), providing limited opportunity for transfer of the active ingredient to naïve nestmates. If contact of treated soil causes workers to cease locomotion and eventually die, and if a substantial number of a colony's foragers tunnel into treated soil, then a colony's worker force could be decimated resulting in severe colony-level impacts and possible colony death. Under the attrition mechanism, colonies that abandon treated soil before experiencing a large loss of workers, for example by favoring alternative food sources away from the structure, could survive treatment. In this regard, it is of interest to note that most colonies in the study area have limited foraging ranges and probably feed on localized food resources (Parman and Vargo 2008). However, one of the surviving treated colonies was foraging on a large loblolly pine stump out in the yard (property BU; Fig. 1), where it persisted for the duration of the study and which presumably served as a large food source throughout the study period. Whether access to this food source was a factor in the colony's survival, we could not determine, nor could we determine whether other surviving colonies had access to similarly large food sources away from the treated area.

The high frequency with which likely treated colonies were redetected in the current study was surprising given the proximity of foragers to soil receiving imidacloprid application (0.3–0.6 m). We expected that colonies this close to the treatment zone would be exposed to the termiticide due to tunneling through treated soil by foragers. Yet, these colonies continued to be detected throughout the study at about the same frequency as the untreated colonies located further away from the application site. Based on these results, we conclude that foragers in these colonies either did not contact treated soil, or did so to such a small extent that these colonies were not strongly impacted. Similar results were obtained by Osbrink et al. (2005) who reported no detectable effect of imidacloprid treatment on *C. formosanus* colonies located 1–3 m from the site of soil application. Based on their results, these authors concluded that imidacloprid treatment did not significantly impact *C. formosanus* colonies. However, unlike the current study, Osbrink et al. (2005) did not include any treated colonies, that is, colonies with observed activity on or immediately adjacent to the foundation wall where the treatment was applied. In our study we found that treated colonies, which were known to receive exposure, were measurably affected by imidacloprid treatment, whereas likely treated colonies were not. Thus it is possible that both in the present investigation and in the study by Osbrink et al. (2005), that many colonies active near but not in the buildings (0.3–3.0 m from the foundation wall) were not exposed to the treatment applied along the foundation wall. Exposure of colonies requires that foragers either be present in the soil at the site of insecticide application or they contact the treated soil after application. Thus, conclusions regarding colony-level effects of liquid termiticide should be based on colonies known to be exposed to treated soil and not just located near the site of application. In addition, such studies should involve tracking exposed colonies over time through genetic markers or other means of colony identification.

There are at least two nonexclusive explanations for the survival of some of the treated and likely treated colonies after imidacloprid application. First, there is reported variation among colonies of *R. flavipes* (Ramakrishnan et al. 2000) and *R. virginicus* (Thorne and Breisch 2001) in susceptibility of imidacloprid exposure applied at or above the label rate of 50 ppm. It is possible that the *R. flavipes* colonies in the current study population varied in imidacloprid susceptibility and that the treatment was not sufficient to kill off the entire populations of the more resistant colonies, even though it was sufficient to eliminate foragers of all colonies from continued feeding on the structures. Second, a previous study showed that workers of *R. virginicus* exposed to even high doses of imidacloprid (100 ppm) for short periods were able to recover, although they showed reduced tunneling activity compared with unexposed nestmates (Thorne and

Breisch 2001). Thus, it is possible that although our treatment killed many of the foragers that contacted the treated soil, in some cases individuals may have moved away from the treated area after encountering it and recovered from exposure.

Although many of the likely treated colonies survived treatment, they were largely driven away from the treatment zone around the structures. We found that activity in the inner ring of monitors, located 0.3–0.6 m from the foundation wall, was greatly reduced over the course of the study, decreasing from 12.5% visitation at the time of treatment to ≈2.5% activity at both the 90- and 730-d time points, a drop of some 80%. Thus, in addition to eliminating subterranean termite colonies from the structure, imidacloprid application seems to discourage colonies from foraging near the treatment zone bordering the foundation wall, reducing the chance of subsequent infestations.

In conclusion, through intensive monitoring of subterranean termites in and around infested structures and by genetic identification and tracking of individual termite colonies, we were able to evaluate the colony-level effects of imidacloprid, a commonly used nonrepellent liquid termiticide, under field conditions. Our results show strong impacts on *Reticulitermes* spp. colonies, often resulting in severe reductions in colony size or colony elimination, although the precise mechanism(s) responsible for this effect remain to be determined. Similar studies of other non repellent liquid termiticides are needed to determine whether they also affect entire colonies.

Acknowledgments

This article is dedicated to the memory of Vincent Parman who tragically passed away 1 January 2007 near the completion of this research. This report is in partial fulfillment of the Ph.D. degree in Entomology at North Carolina State University, where he was in the final stages of his graduate studies in the Department of Entomology at North Carolina State University at the time of his death. We thank Joshua Jordon for help in installing the monitors, collecting samples, and performing most of the genetic analyses. Paul Labadie gave expert assistance in genotyping and analyses. This work was generously supported by funding from Bayer Environmental Science.

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Received 13 November 2009; accepted 6 March 2010.