Elimination of Subterranean Termite (Isoptera: Rhinotermitidae) Colonies Using a Refined Cellulose Bait Matrix Containing Noviflumuron When Monitored and Replenished Quarterly

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ABSTRACT Using a quarterly (3-mo) monitoring and bait-replenishment interval, 122 subterranean termite colonies throughout the United States were baited with a refined cellulose bait matrix containing 0.5% noviflumuron. All colonies were eliminated in less than 1 yr after initiation of baiting as determined by long-term monitoring and genetic markers. Sixty-three percent of the colonies were eliminated during the first quarter after the initiation of baiting and 77% of colonies were eliminated after consuming two bait tubes or less. This suggests that a single baiting cycle and bait installed in response to a single active monitoring device were sufficient to eliminate the majority of colonies. Although termites temporarily abandoned stations after depleting bait, workers resumed feeding when baits were replenished. Colonies that consumed large amounts of bait before elimination foraged into multiple stations, thus allowing adequate amounts of bait to sustain feeding. The time to eliminate termite colonies with bait replenished quarterly was similar to that previously reported for laminated cellulose bait replenished monthly. Our data support the conclusion that extending the bait replenishment interval from monthly to quarterly for bait tubes with refined cellulose containing 0.5% noviflumuron did not adversely impact colony elimination.

KEY WORDS termite baiting, noviflumuron bait, colony elimination, subterranean termite

In 1994, hexaflumuron, a benzoylphenyl urea chitin synthesis inhibitor, was the first active ingredient registered in the United States for subterranean termite control in a bait formulation (0.1% active ingredient [AI], Recruit, Dow AgroSciences LLC, Indianapolis, IN) (Robertson and Su 1995). Hexaflumuron, formulated on wood powder as Recruit or later on a laminated cellulose bait matrix as Recruit II (0.5% AI, Dow AgroSciences LLC), was tested globally for termite colony or population elimination. Su (2003) provided a summary of 33 published studies with elimination of 152 of 159 baited colonies using hexaflumuron baits. Using molecular genetic markers, Vargo (2003a) documented the elimination of 36 colonies of *Reticulitermes* spp. also treated with hexaflumuron bait. Monitoring devices and baits in these trials were usually evaluated monthly and replenished as needed at monthly intervals.

Longer service intervals would be beneficial to reduce the cost of service visits by pest management professionals (PMPs). Further, less frequent monitoring would reduce the cost of baiting. Frequent servicing may occasionally cause termites to abandon stations if they are sensitive to disturbance (Swoboda et al. 2004). Less frequent disruption of stations may result in reduced station abandonment and increased bait consumption, resulting in better control of subterranean termites. To provide similar control with less frequent service intervals, bait systems would have to deliver more bait, increase the toxicity of the bait, or both.

In 2004, noviflumuron, 0.5% AI on a laminated cellulose matrix (Recruit III, Dow AgroSciences LLC) was registered for termite control in the United States. Noviflumuron is a chitin synthesis inhibitor, is readily consumed by subterranean termites and is efficiently transferred to untreated termites (Karr et al. 2004). However, noviflumuron is retained in termites much longer with a half-life of 29 d versus 8–9 d for hexaflu-

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Table 1. Termite species, number of colonies and states included in a quarterly monitoring/bait replenishment study, 2004–2006

Species tested	No. colonies baited	States where tests were conducted
C. formosanus	5	Hawaii, Louisiana
H. aureus	4	Arizona
R. flavipes	72	Florida, Georgia, Iowa, Indiana, Mississippi, North Carolina, Ohio, Tennessee, Texas, Virginia
R. hageni	22	Florida, Georgia
R. hesperus	9	California
R. virginicus	10	Florida, Tennessee

muron and it is two- to three-fold more toxic than hexaflumuron (Karr et al. 2004). Smith et al. (2002) reported on the elimination of 53 colonies with hexaflumuron and 74 colonies with noviflumuron and concluded that the number of days required for colony elimination with noviflumuron was about half that with hexaflumuron. Other studies have demonstrated elimination of subterranean termites with noviflumuron bait (Getty et al. 2007, Husseneder et al. 2007, Austin et al. 2008).

When noviflumuron was introduced, the required monitoring/bait replenishment interval was increased to every other month because of its higher intrinsic activity. In 2004, a new refined cellulose bait matrix containing 0.5% noviflumuron (Recruit IV, Dow AgroSciences LLC) was developed. This matrix is about twice as dense as the laminated cellulose matrix and provides almost double the amount of noviflumuron available to termites in a same size bait tube. This study was initiated in 2004 with the hypothesis that Recruit IV (0.5% noviflumuron) with quarterly monitoring and bait replenishment will eliminate subterranean termite colonies.

Methods and Materials

Sites. In total, 122 colonies were baited under a quarterly monitoring/bait replenishment protocol in 2004–2006. Table 1 provides the species tested, number of colonies baited for each species and the states where studies were conducted. Trials were conducted in areas of known termite activity. Test sites consisted primarily of structures, but three grid sites away from structures were also used. Commercial monitoring stations (Sentricon Termite Colony Elimination System, Dow AgroSciences LLC) were installed around structures according to label directions. In grid sites, monitoring stations were installed and mapped for subsequent inspection. Sites with multiple active stations were targeted for these studies although a number of structures had a single active station. At grid sites, only termite colonies infesting three or more stations were baited. Two-thirds of these stations were baited, while monitoring devices were left in the other third to serve as untreated controls.

Termite Colony Characterization. When multiple active stations were present in early studies, colonies were characterized using bait tubes containing a cellulose matrix impregnated with one of the following dyes: Nile Blue A (0.1% wt:wt), Neutral Red (0.5% wt:wt), or a combination of Nile Blue (0.05% wt:wt) and Neutral Red (0.25% wt:wt) that yielded a purple color. Methods described by Atkinson (2000) were used to determine foraging territories of individual subterranean termite colonies using these dye bait tubes. Regardless of dye characterization, colonies of *Coptotermes formosanus* Shiraki and all *Reticulitermes* spp. were characterized by genetic structure using microsatellite markers (Vargo 2003a,b; Husseneder et al. 2007). Ten termites were genotyped at each of several microsatellite loci. Loci typically used were: *Rf 24–2*, *Rf 21–1*, *Rf 6–1*, and *RS1* for eastern *Reticulitermes* spp.; *RS1* and *Rf 21–1* for western species of *Reticulitermes;* and *Cf 8–4*, and *Cf 12–4* for *C. formosanus.* At least two loci were generally used for each sample, but if relationships were not clear, additional termites were genotyped at other loci. Details of the extraction and genotyping procedures are provided by Dronnet et al. (2004), Vargo (2003a,b), and Vargo and Henderson (2000). Microsatellite markers had not been identified for *Heterotermes aureus* (Snyder) so these colonies were only characterized using dyed bait tubes. Termites were collected from structures and active stations, stored in 95% ethyl alcohol for molecular characterization and confirmation of species determinations. Termites found in stations after elimination or in newly active monitoring stations were also collected and characterized to determine whether these new termites were from the original colony or represented a new infestation.

Termite Monitoring and Baiting. After installation of stations, sites were monitored monthly for termite activity. However, all service changes to the system such as installation of additional stations or installation and replacement of monitoring devices or baits were made only at 3-mo intervals to simulate a quarterly monitoring/replenishment schedule. The purpose of monthly monitoring was to more accurately estimate time to elimination than was possible if stations were only monitored quarterly. Data collected at monthly visits were the numbers of termites present and a visual estimate of percent consumption of bait or monitoring devices.

Baiting was initiated after active monitoring stations were found. Bait tubes containing 65 g of 0.5% noviflumuron on a refined cellulose bait matrix were placed in stations with live termites at quarterly intervals. At the time of baiting, two auxiliary stations were installed within 30.48 cm of the primary station if suitable ground access was available. One auxiliary station contained a bait tube and the other contained a wood monitoring device. Wood monitors in close proximity to the active station served as a nontoxic food source to evaluate continued termite foraging in the vicinity of the baited station (Su and Scheffrahn 1996). Before installation, bait tubes were moistened with 30–90 ml of water or a sports drink such as Gatorade (Pepsico, Purchase, NY). Termites collected from the active station were placed inside the bait tube

and on top of the bait tube after insertion of the bait tube into the station. When more termites than would fit into a single bait tube were available, they were also added to the baited auxiliary station.

Termite colonies were considered eliminated from a site when there were no live termites present in connected stations and no additional bait was consumed for two successive monthly evaluations. If a structure was infested, elimination of the colony from that structure was also required to consider the colony eliminated. The date of elimination was considered to be the first monthly evaluation date on which no live termites were present in the structure and no live termites or additional bait consumption were noted in any of the connected stations. Studies were continued for 6 mo to 1 yr after elimination to monitor for reinfestation. Structural inspections were conducted at trial initiation, at the time of colony elimination from stations and at 6–12 mo postelimination. In addition to a flashlight and probing tool, inspections were conducted using a moisture meter or an inspection tool that used microwave, acoustic detection, or infrared sensing technology. Some of the trials reported here were conducted under requirements for state registration in Florida and results discussed by Thoms et al. (2009).

Elimination time and bait consumption for baiting initiated in April to August or September to March were analyzed using a Kruskal–Wallis test to compare different time regimes. Data for infested and noninfested structures and for different species were also compared using Kruskal–Wallis. Bait consumption in primary active stations versus auxiliary stations were compared using a paired *t*-test. The analytical software used was Minitab (State College, PA).

Results and Discussion

Elimination Time and Bait Consumption. All 122 colonies were eliminated in less than 1 yr after bait installation. The time for colony elimination and the amount of bait consumed before elimination are summarized by species in Table 2. The median number of days to elimination was within the first quarter (0–91 d) after bait installation for all species except *H. aureus.* The median number of days to elimination for this species fell within the second quarter at 136 d. The variation in days to elimination was quite large for all species and probably reflects differences in the time of year that baiting was initiated and/or differences in colony size or foraging area. When data from all species were combined, the mean (96 d) and median (74 d) to elimination with quarterly bait replenishment were very similar to those reported by Smith et al. (2002) for the same complex of species when bait was replenished monthly (107 and 90 d, respectively).

Nearly all colonies baited consumed the equivalent of 0.1 bait tubes or more, but three colonies consumed the equivalent of only ≈0.02 bait tubes or a little over 1 g of bait. Two of these were *H. aureus* and one was *R. hageni* Banks. These colonies may have been eliminated or may have abandoned the sites. Because we did not detect them during subsequent station inspections, we assumed that these colonies were very small and were eliminated by the small amount of bait consumed.

Table 2. Termite species, elimination time, and bait consumption for subterranean termite colonies in a quarterly monitoring/bait replenishment study, 2004–2006

Species	No. colonies	Mean[a]	Median	Range	SE
Days to elimination					
C. formosanus	5	137a	91	56–266	39
H. aureus	4	128a	136	24–215	50
R. flavipes	72	99a	86	20–342	9
R. hageni	22	70a	62	20–232	11
R. hesperus	9	113a	76	33–334	32
R. virginicus	10	82a	46	20–290	28
All Species	122	96	74	20–342	7
Bait consumed (grams)					
C. formosanus	5	296a	40	10–1,170	222
H. aureus	4	7b	7	1–13	3
R. flavipes	72	149a	90	13–1,433	24
R. hageni	22	31a	25	1–163	7
R. hesperus	9	61a	59	7–178	20
R. virginicus	10	68a	36	10–345	32
All species	122	116	60	1–1,433	18

[a] Means followed by the same letter in a column are not significantly different ($P = 0.05$) according to a Kruskal–Wallis Test.

The mean time to elimination for each species tested fell within the first quarter (0–91 d) for at least 50% of the baited colonies (Fig. 1). When data from all species were combined, 63% of colonies were eliminated in the first quarter. Twenty-two percent of colonies were eliminated in the second quarter after initiation of baiting, 10% of colonies were eliminated in the third quarter and only 5% required a fourth quarter for elimination.

Mean and median bait consumption was equivalent to or less than the amount contained in a single bait tube (65 g) for all species except *C. formosanus* and *R. flavipes* (Kollar) (Table 2). Each of these species had much higher average bait consumption. They also had a large range in bait consumption, from ≈0.2 bait tube equivalents to 18 and 22 bait tube equivalents, respectively. A bait tube equivalent is 65 g of consumption

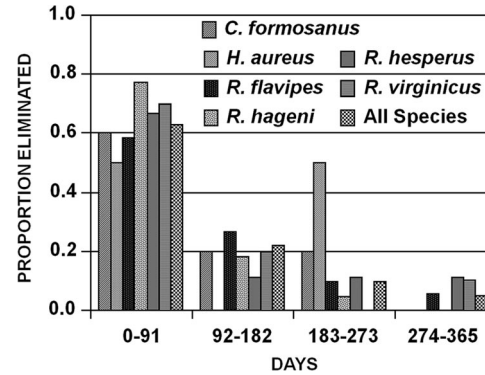

Fig. 1. Proportion of colonies eliminated in each quarter after initial bait installation for six species of subterranean termites.

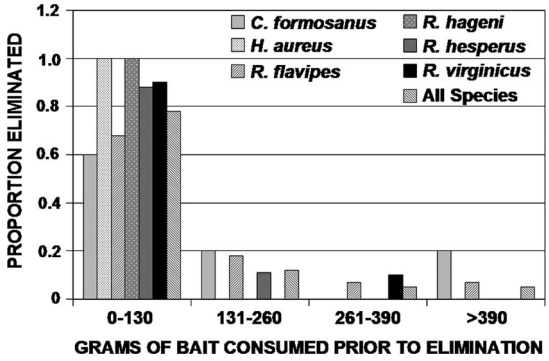

Fig. 2. Proportion of subterranean termite colonies consuming different quantities of bait before elimination. Data are grouped by bait tube equivalents consumed (0–130 g = 1–2 bait tubes, 131–260 = 3–4 bait tubes, etc.).

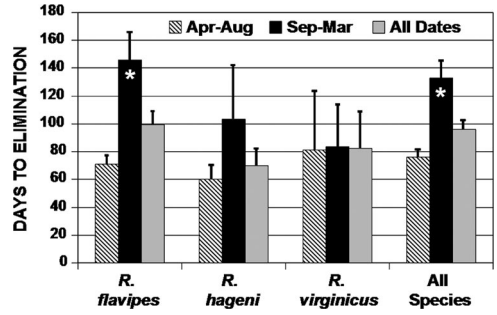

Fig. 3. Effect of timing of initial bait installation on mean days to colony elimination. Error bars represent SEM. An asterisk in the Sep–Mar bar indicates that the two timings were not statistically similar (Kruskal–Wallis, $P = 0.05$).

that may be complete consumption of a single bait tube or partial consumption of bait across several bait tubes that adds up to 65 g. For example, 25% consumption in four bait tubes would add up to a single bait tube equivalent. The two colonies consuming the largest amounts of bait were among those with the longest elimination times and will be discussed individually. *H. aureus* had the lowest bait consumption and the range was relatively small. This may be because of the small sample size of only four colonies. Mean consumption by this species was significantly lower than that for all other species. No other significant differences in bait consumption were observed among species. Among species of *Reticulitermes,* consumption was numerically lowest for *R. hageni* Banks and highest for *R. flavipes. R. hesperus* Banks and *R. virginicus* (Banks) consumed similar amounts of bait. The variability in bait consumption was relatively low for all species except *C. formosanus* (Table 2). Two of the five colonies of *C. formosanus* that were eliminated consumed relatively large amounts of bait, hence the large variation. The other species were fairly consistent in the amount of bait consumed before colony elimination although one colony of *R. flavipes* consumed a large quantity of bait and eleven colonies consumed >260 g, the equivalent of four bait tubes.

The proportion of colonies consuming 130 g of bait or less before elimination was 60% or higher for each of the species tested (Fig. 2). When data for all species were combined, nearly 80% of colonies were eliminated after consuming 130 g or less. This number is significant because 130 g is equivalent to two bait tubes, the number that would be installed in response to a single active monitoring device (one bait tube replacing the monitoring device and one installed in an auxiliary station). Only 23% of colonies consumed >130 g before elimination, most of those being *C. formosanus* and *R. flavipes.* Thus, when a single monitoring station was active, bait provided in that primary station plus an auxiliary station was enough to eliminate most colonies.

Timing of Bait Installation. The time of year when baiting is initiated can play an important role in the time needed to eliminate a colony, particularly in colder climates (Smith et al. 2002; Spomer and Kamble 2005, 2006). For *R. flavipes,* the days to colony elimination was over twice as long for colonies baited from September to March than for those baited from April to August (Fig. 3). This difference was significant ($P = 0.001$). *R. hageni* also required considerably more time to reach colony elimination when baiting was initiated in the September to March time frame, but the difference was not significant ($P = 0.158$). There was little difference in days to elimination for the two bait installation timings with *R. virginicus.* When data for all species were combined the difference was again significant ($P = 0.001$) although the large number of *R. flavipes* trials tended to bias results summarized across species. Data on the effect of bait timing are not presented for *C. formosanus, H. aureus,* or *R. hesperus,* because of small sample size or the initiation of all trials during only one of the April to August or September to March time frames.

The amount of bait consumed by *R. flavipes* was ≈30% higher for colonies first baited in the September to March time frame than for those baited from April to August (Fig. 4). When data were combined across all species, consumption was also ≈30% higher for the September to March baiting time frame, again probably reflective of the dominance of *R. flavipes* in the data set. *R. hageni* and *R. virginicus* consumed lower

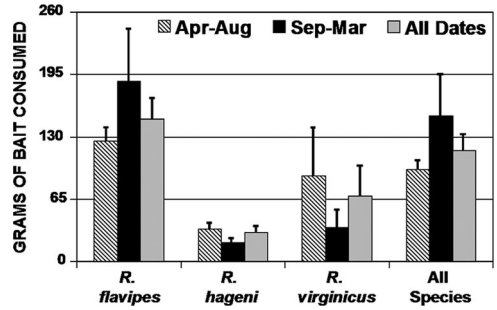

Fig. 4. Effect of timing of initial bait installation on bait consumption. Error bars represent SEM. There were no significant differences in means (Kruskal–Wallis, $P = 0.05$).

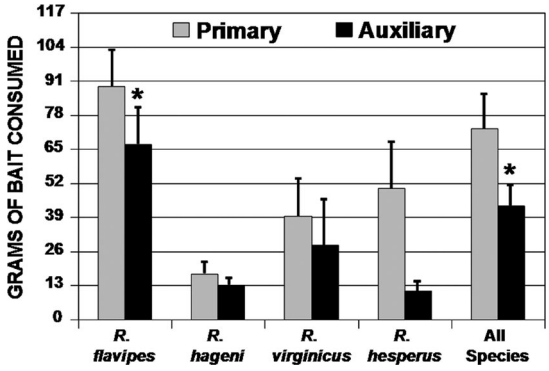

Fig. 5. Mean bait consumption in primary and auxiliary stations. Error bars represent SEM. An asterisk above the auxiliary bar indicates that the two station types were not statistically similar (paired *t*-test, $P = 0.05$).

amounts of bait when baited in September to March than when baited in April to August. The difference was small for *R. hageni*, but *R. virginicus* consumed less than half as much bait when baited in the September to March timing. However, the differences in consumption were not significant ($P = 0.05$).

The longer time needed for colony elimination during cooler times of the year is not surprising. The primary mode of action of noviflumuron is interference with ecdysis (Su and Scheffrahn 1993), which occurs less frequently at lower temperatures. Spomer and Kamble (2006) showed that transfer of noviflumuron from treated to nontreated workers was slower at lower temperatures. They also reported that uptake of noviflumuron and clearance from termite workers is slower at lower temperatures. Thus, longer colony elimination times would be expected when baiting occurs under cooler conditions. Because consumption is greater over a shorter time at warmer temperatures and lower over a longer time at cooler temperatures, total consumption necessary for colony elimination should be similar, regardless of temperature. Our results support this assumption as there were no significant differences in consumption of bait for the different bait installation times.

Performance of Auxiliary Stations. Differences in bait consumption in primary versus auxiliary stations are illustrated in Fig. 5. Data for *H. aureus* and *C. formosanus* are not included in the graph because of small sample size and because stations in many of the trials with *C. formosanus* were installed through cores drilled in concrete and auxiliary stations were not installed. While all species consumed more bait in primary stations than in auxiliary stations, the difference was significant only for *R. flavipes* ($P = 0.003$) and for all species combined ($P = 0.01$). Despite these differences, total bait consumption was significantly higher for primary + auxiliary stations combined than for primary stations alone for all species combined ($P < 0.001$), *R. flavipes* ($P < 0.001$), *R. hageni* ($P < 0.001$), and *R. hesperus* ($P = 0.027$). Thirty-six percent of all bait consumed by *R. flavipes* and 38% of that consumed by all species combined was consumed in auxiliary stations. Paysen et al. (2004) found that use of auxiliary stations increased bait consumption by *Reticulitermes* spp. by 41%, results similar to ours. They also found that the use of auxiliary stations improved the ability to maintain termite activity in primary stations by 36%. These results suggest that installing and baiting auxiliary stations is the best practice to increase the amount of bait consumed when termite activity is detected in stations.

Performance in Infested Versus Noninfested Structures. There were 62 structures included in these trials and 104 colonies were eliminated at the structures. Nineteen of the structures were infested and 30 colonies were eliminated at these structures. Forty-three of the structures did not have termites in the structure, but termites were present in monitoring stations around the structures. Seventy-four colonies were eliminated around these structures. All structural infestations were eliminated in trials included in this study. Colony elimination required 15 d longer in infested structures than in noninfested structures (117 and 102 d, respectively), but the differences were not significant ($P = 0.054$). Bait consumption was significantly higher for colonies at infested structures ($P = 0.013$) with a mean of 161 g consumed as compared with 111 g consumed at noninfested structures. Species infesting structures were usually *C. formosanus* and *R. flavipes.* These species generally consumed more bait before elimination than did the other species and their predominance at infested structures may explain the higher consumption rates at infested versus noninfested structures.

Individual Sites. A few sites were clearly unique or demonstrated key observations and warrant elaboration here. The first of these sites was infested by a colony of *R. flavipes* that consumed 1,433 g of bait (22 bait tubes). The colony was located around a wooden pole barn in Riverview, FL. Figure 6A shows the distribution of stations around this structure. Twelve monitoring stations were installed around this structure and activity was observed in nine of the stations in August 2004. At that time, one red and one blue dye bait tube were placed into stations on opposite sides of the structure. At 1 and 2 mo after installation of the dye matrix, there was a small amount of consumption of the blue dye matrix and blue termites were found, but only in the station with the blue dye. The red dye matrix was completely consumed at 1 mo and the station was abandoned. No red-dyed termites were found in any of the stations at any time during the course of the study suggesting that the colony was very large and widely dispersed. In November, seven stations remained active and samples were collected for genetic characterization. The seven active stations were baited and two auxiliary stations installed for each, one provided with bait and the other with a monitoring device. One month after installation, 11 of the 14 bait tubes were completely consumed, and all bait was consumed by 2 mo after installation. At the 3-mo inspection, the 14 bait tubes previously consumed were replaced and another of the original monitoring

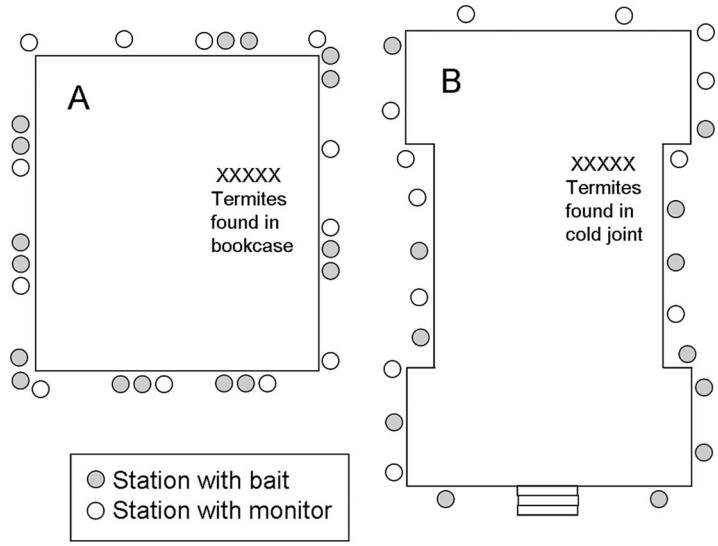

Fig. 6. Diagram of structures in Riverview, FL (A) and New Orleans, LA (B) showing placement of stations containing bait and monitors. Auxiliary stations were not installed in the New Orleans structure because all stations had to be installed through concrete.

stations was active, so a total of 16 new bait tubes were installed. One month later, every bait tube demonstrated some level of consumption and termites were found in all of the bait tubes. Consumption continued, but at the 6-mo replenishment inspection only two bait tubes were completely consumed and activity was no longer detected in several of the stations. At this 6-mo inspection, 12 additional baits were installed. Feeding on these was minimal and termites were eliminated in June, 2005, 220 d after baiting was initiated. Genetic analysis confirmed that all termites collected from this location were from a single colony.

The second example was a colony of *C. formosanus* that consumed the equivalent of 18 bait tubes (1,170 g). The colony was located around an infested masonry office building in New Orleans, LA (Fig. 6B). Twenty-four monitoring stations were installed around the structure through holes drilled in concrete. Auxiliary stations were not installed at this site because of the lack of soil access. At the initiation of baiting, three stations were active and each was baited. Two of these bait tubes were completely consumed at 1 mo after installation and all three were consumed at 2 mo postinstallation. Bait was replenished in these three stations at the quarterly inspection. At this time, eight additional stations were active and all of these were baited for a total of 11 baited stations. Of the 11 baited stations, 8 were completely consumed before the 6-mo replenishment inspection and one new monitoring station was active. Twelve new baits were installed at the 6-mo inspection. At 9 mo after baiting was initiated, four additional bait tubes were completely consumed, but no live termites were detected and the colony was eliminated 266 d after bait initiation. All termites collected from active stations and inside the structure were genetically similar and presumed to belong to the same colony.

These two examples represent the extreme of bait consumption in trials conducted for this study. The next highest consumption for *C. formosanus* was 176 g (2.7 bait tubes). Except for the previous example, none of the other colonies of *R. flavipes* consumed >621 g (9.6 bait tubes). Although these colonies are of interest because of the extremes in bait consumption, they also illustrate two important observations made in a number of trials. First, even though many of the bait tubes were completely consumed before the quarterly replenishment, in some cases 2 mo prior, termites returned to all of these stations and continued to feed once new baits were installed. One of the concerns with quarterly replenishment was that termites would abandon stations once all of the bait was consumed. We found that while termites may temporarily abandon stations once the bait is exhausted, they continued to forage in these stations and returned when new bait was introduced as needed at quarterly intervals. This occurred even when stations were disturbed monthly for inspections required by the research protocol. A second concern is that large colonies may not get enough bait if they are feeding at single stations and bait is not replaced monthly or every other month. As these examples illustrate, colonies that required large amounts of bait for elimination were usually widespread and foraged into multiple monitoring stations. This allowed delivery of adequate quantities of bait for these colonies, even when replenished quarterly.

In summary, we found that quarterly replenishment of bait tubes with refined cellulose noviflumuron bait did not appear to adversely impact colony elimination, time to elimination or bait consumption compared with previously published monthly replenishment data (Smith et al. 2002). Termites temporarily abandoned stations when bait was completely consumed,

but returned and resumed feeding when new bait was installed. Colonies that consumed large amounts of bait before elimination foraged into numerous monitoring stations, thus providing additional opportunities for delivery of adequate amounts of bait. Concerns that quarterly monitoring would result in permanent abandonment of baited stations if bait was replenished quarterly or that quarterly replenishment would not provide adequate amounts of bait were not supported by our research. Results from these studies suggest that key structure-infesting subterranean termite species found in the United States are readily eliminated using refined cellulose noviflumuron bait on a quarterly monitoring/bait replenishment schedule.

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