

Response of *Reticulitermes hesperus* (Isoptera: Rhinotermitidae) Colonies to Baiting With Lufenuron in Northern California

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ABSTRACT The objective of this study was to evaluate lufenuron termite bait (1,500 ppm) for the elimination of colonies of *Reticulitermes hesperus* Banks (Isoptera: Rhinotermitidae). Dispersion of colonies in six baited and six unbaited sites near Placerville, CA, was determined by genetic (microsatellite) analyses. Twenty-one colonies of *R. hesperus* inhabited the six baited sites and eight colonies of *R. hesperus* occurred in the six unbaited sites. Five criteria provided a cause-and-effect link between the deployment of lufenuron termite bait and elimination of baited colonies: 1) association of foragers, as members of the same colony, in the independent monitoring stations and bait stations; 2) quantity of bait consumed; 3) abnormal physical appearance of foragers in bait stations; 4) disappearance of foragers from, and cessation of feeding in, independent monitoring stations visited by baited colonies; and 5) presence of foragers from, and continuation of feeding in, independent monitors visited by unbaited colonies. Baited colonies were devoid of foraging termites within a mean of 70.6 d (range, 37–93 d) of bait deployment. Colonies consumed a mean of 8.0 g of bait (range, 2.2–16.0 g). Wood consumption by baited and unbaited colonies was not significantly different during the 2 mo before baiting, 281.4 versus 590.5 mg/d per colony, respectively, nor during the 3 mo immediately after baiting, 112.5 versus 436.8 mg/d per colony, respectively. However, from 10 to 16 mo after baiting, wood consumption by baited colonies essentially ceased and was significantly less than the unbaited colonies, 7.9 versus 470.1 mg/d per colony, respectively.

KEY WORDS benzoyl urea insect growth regulator, chitin synthesis inhibitor, subterranean termites, termite baits, termite colony elimination

The use of baits for the control of subterranean termites has been a goal for decades (Randall and Doody 1934), with a significant research effort since the 1970s (Esenther and Beal 1978; Su 1994; Forschler and Ryder 1996; Haagsma and Bean 1998; Su and Scheffrahn 1998; Getty et al. 2000b, 2007; Jones 2003; Messenger et al. 2005). The success of termite bait applications is dependent on bait consumption, knowledge of the toxicity and mode of action of the active ingredient and an understanding of the foraging behavior of the targeted species (Thorne and Forschler 2000, Grace and Su 2001). Many variables, including seasonal weather patterns, predation, competition between nearby colonies, size of a colony, density of colonies, disturbance, and available, alternative food sources can affect visitation by foragers to monitoring and bait

stations and thus confound the interpretation of bait efficacy (Forschler and Ryder 1996, Haverty et al. 1999b).

Because of the cryptic life style of subterranean termites, assessment of bait efficacy can be difficult. From a research perspective, it is critical to have an independent monitoring system, separate from a bait station with bait in it, for observing the effects of baits on termites. Because termites may return to a successfully baited area, a distinction must be made between foragers from a baited colony and those from invading, neighboring colonies to determine whether colony elimination has been achieved (Getty et al. 2000a, Messenger et al. 2005). Independent monitoring stations inhabited by foragers from the same colony as those visiting a bait station, become important tools for observing the effects of baiting (Lewis et al. 1998, Getty et al. 2000b).

In laboratory studies lufenuron-treated paper or wood sawdust caused significant mortality in *Reticulitermes flavipes* (Kollar) and *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) (Su and Scheffrahn 1996b, Vahabzadeh et al. 2007). Lewis and Power (2006) demonstrated that lufenuron (1500 ppm) resulted in mortality equivalent to noviflumuron (5,000 ppm) in laboratory tests against *Reticulitermes*

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hesperus Banks. In 2004, lufenuron was registered by the U.S. Environmental Protection Agency for control of subterranean termites. Herein we report the results of a field experiment to evaluate lufenuron in a bait for control of colonies of *R. hesperus*. We document control of baited colonies followed by immigration of new colonies into vacated foraging territories. In light of these results, we discuss the complexity of proving colony elimination against subterranean termites.

Materials and Methods

Study Locations. The field location was the Eddy Arboretum of the USDA Forest Service, Pacific Southwest (PSW) Research Station's Institute of Forest Genetics (IFG) in Placerville, CA. The Eddy Arboretum has been used in previous studies of *Reticulitermes* chemical taxonomy (Haverty and Nelson 1997, Copren et al. 2005), behavior and population ecology (Haverty et al. 1999a,b, 2000; Delphia et al. 2003), and control with baits (Getty et al. 2000b). Within this 4-ha arboretum independent monitoring stations (IMs) were previously established and were used by numerous colonies of *Reticulitermes* (Lewis et al. 1998), most of which were *R. hesperus* (Haverty and Nelson 1997; Haverty et al. 1999b, 2003).

Termite and wood samples collected from the field were processed at the PSW Forestry Sciences Laboratory in Albany, CA, and the University of California's Richmond Field Station in Richmond, CA.

Establishment of Independent Monitors and SecureChoice Monitoring Stations. In June 2003, we selected 12 IMs that contained numerous *R. hesperus* foragers and measurable wood consumption. These IMs were dispersed throughout the arboretum and were far enough apart (>8 m) to ensure that foragers were from different colonies. Treatments were randomly assigned to each IM; six were lufenuron-baited and six were left unbaited.

From June to August 2003, 24 Douglas-fir (*Pseudotsuga menziesii* [Mirb.] stakes (2.5- by 5- by 30-cm) were spaced around each of the 12 IMs in a radial grid with three placed at 1-m intervals in each cardinal and intermediate (eight) directions (see Figs. 1 and 2). Stakes were monitored for termite activity, and stakes with ≥ 20 *R. hesperus* foragers and $\geq 20\%$ consumption of the stake below-ground were used as focal points to install one or two additional IMs (Figs. 1 and 2). At each of the 12 sites, a primary IM was selected and around each of these primary IMs eight SecureChoice monitoring stations (MSs) (Syngenta Crop Protection, Inc., Greensboro, NC) were placed at 0.5 and 1.5 m from the IM in each of four cardinal directions (Figs. 1 and 2). All additional IMs and MSs were installed by December 2003.

Determination of Foraging Territories and Colony Affiliation. By 17 December 2003, each site was established with a primary IM, at least one secondary IM, and eight MSs. Termite activity in all stations and wood consumption in IMs were monitored monthly for 7 mo. For the sites assigned to the baiting treatment, we established an affiliation among termites in

the IMs and termites in one or more of the MSs. These affiliations were based on microsatellite analyses. Affiliated foragers were assigned to colonies and each colony was uniquely labeled with uppercase letters (A, B, C, D, and/or E) for each of the 12 sites.

Samples of 20–30 workers were collected when possible and uniquely identified by IMs or MSs and date for analysis of microsatellite loci. The analyses were based on five individuals per sample and genotyped for four microsatellite loci (*Rf6-1*, *Rf5-10*, *Rs10*, and *Rs78*) according to the methods of Vargo (2000) and Dronnet et al. (2004). For each site, forager genotypes from all of the sampled stations were compared by means of a log likelihood exact test of genotypic differentiation as implemented in the program Genepop (Raymond and Rousset 1995; available at <http://genepop.curtin.edu.au/index.html>). Samples that differed significantly ($P < 0.05$) at one or more loci were considered to belong to different colonies as described in previous studies of subterranean termites (DeHeer and Vargo 2004, Vargo et al. 2006).

Wood Consumption as a Measure of Termite Activity Within a Colony. Monthly wood consumption in IMs was the primary indicator of termite activity with implications of colony viability over time. Wooden bundles in the IMs were used for the feeding substrate and each bundle consisted of 12 pieces (30- by 3- by 1-cm) of Douglas-fir. Two bundles were assigned to each IM that allowed deployment of one in the IM, whereas the other was processed to determine wood consumption, as described previously (Haverty et al. 1999b). Wood lost in IMs with no termite activity was measured to correct for wood consumption by baited colonies and served as a measure of the error in our techniques for determining wood consumption (Getty et al. 2000b). During bundle assessment, foragers were sampled as needed for bioassays or returned with the replacement bundle.

Delivery of Lufenuron Termite Bait, Measurement of Bait Consumption, and Time to Colony Elimination. At the six sites assigned to the lufenuron bait treatment two to three MSs with consistent termite activity (≥ 20 foragers) and wood consumption were selected for baiting. In the MSs selected for baiting, the pressed cellulose liner tube and monitoring stick were removed and a lufenuron termite bait tube was deployed on 20 July 2004.

Each bait tube consisted of a clear, plastic cylinder (5.0 cm in diameter, 21 cm in length) with a conical tip. Termite forager access to the bait matrix was through 0.5-cm-diameter holes drilled into the cylinder (five holes in the tip and eight rows of 15 holes along the cylinder wall). Lufenuron [*N*-[2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)-phenylaminocarbonyl]-2,6-difluorobenzamide], a benzoyl urea insect growth regulator (IGR), was the active ingredient in the bait matrix, which was comprised of ≈ 21.2 g of corrugated paper impregnated with lufenuron at 1,500 ppm. MSs containing a bait tube were monitored monthly to observe presence of termites and estimate the amount of bait consumed.

Bait tubes were removed and replaced with a new bait tube when visually >50% of the bait was consumed. When ants were present, and displaced termites, the bait tube was replaced with a monitoring stick. Partially consumed bait tubes were returned to the laboratory and opened to remove the treated paper, which was then cleaned, dried, and weighed. The amount of bait consumed during deployment was calculated using the average dry weight of treated paper in 10 unused baits as the initial dry weight before deployment minus the dry weight of the remaining portion of the treated paper after removal of the bait.

Unequivocal proof of colony elimination in the field was not possible. However, colony elimination in this study was defined based on a comparison of criteria measured before, during and after baiting relative to nonbaited colonies. So based on before baiting assessments, colonies were defined as being eliminated with 1) continual absence of foragers from the colony feeding on the bait and 2) the significant reduction, or cessation, of wood consumption by that colony (Getty et al. 2000b, Ripa et al. 2007). Furthermore, when coupled with observations (four of six colonies) of the symptoms of benzoyl urea poisoning (Su and Scheffrahn 1996a), we concluded that the colonies were eliminated due to consumption of the bait. Lastly, because forager mortality was not quantifiable during baiting, the time required to eliminate a colony was estimated. We used the number of days from bait deployment to the midpoint between the last day post baiting that live foragers were seen in the IM(s) and baited MSs, and the first day postbaiting when no live foragers were seen in the IMs or MSs.

Statistical Analysis. The amount of wood consumed in the IMs by a colony on a daily basis before, during, and after baiting was important to addressing the objectives of this study. This evaluation continued for over 15 mo, from 3 June 2004 until 15 November 2005. This period was divided into four events: 3 June 2004 through 10 August 2004 (before baiting), 10 August 2004 through 5 November 2004 (post baiting I), 5 November 2004 through 3 May 2005 (the seasonal doldrums when little or no feeding occurred), and 3 May 2005 through 15 November 2005 (post baiting II). The mean amount of wood consumed (mg/d) for each colony during each of these four events was used for the statistical analysis.

To quantify the effect of lufenuron on a colony, wood consumption in IMs present at a baited site and used by colonies that did not feed on bait were included with wood consumption of colonies at the unbaited sites. This was done as there was no reason to believe that they would have different characteristics from the colonies in the original unbaited sites. As a result our sample consisted of five baited colonies and 12 unbaited colonies.

A log ($X + 1$) transformation of the weight change was used to normalize wood consumption before statistical analysis. A repeated measures analysis of variance (ANOVA) (SAS Institute 2004) was performed on the transformed weight-change variable using the

values for the before baiting, post baiting I, and post baiting II time periods.

Results and Discussion

Number of Colonies and Foraging Territories at Each Site. Twenty-six IMs were occupied by *Reticulitermes* at baited (16 IMs) and unbaited (10 IMs) sites. Twenty-three of the IMs were occupied by *R. hesperus*, whereas three IMs (one at site 4 and two at site 12) were used by foragers characterized as cuticular hydrocarbon phenotype CA-B, an undescribed species of *Reticulitermes* (Copren et al. 2005, Nelson et al. 2008). Before baiting, our primary focus was to understand the affiliations of the IMs and MSs within sites that were to be baited. This was done using microsatellite analyses. After baiting, we also relied on microsatellite analyses to ascertain which active IMs and MSs were used by the same colonies and which colonies were eliminated.

Demonstration of colony elimination requires a definition of a termite colony. Because the bait toxicant can only be distributed within a colony via foragers moving within an interconnected gallery system, the interconnectivity of IMs and MSs is an important issue (Su and Scheffrahn 1998). We assumed that foragers in IMs and MSs with similar microsatellite genotypes belonged to the same colony, even though this assay does not unequivocally demonstrate that the foragers are moving within an interconnected gallery system. Traditional mark-release techniques have been used to document interconnectedness of bait/monitoring stations, but even this technology can falsely report the lack of connection among monitoring stations within a colony (Baker and Haverty 2007).

Baited Sites (1–6). At site 1 four colonies (A–D) were identified as occupying three IMs and three MSs before baiting (Fig. 1). After baits were deployed foragers from colony B disappeared by 5 November 2004. Lufenuron termite bait eliminated colony B; however, colony A remained until the close of the study in January 2006. Colonies C and D were found only before baiting. One year after baiting was initiated, a new colony (colony E) appeared in one of the MSs (Fig. 1).

At site 2 one IM and four MSs that were occupied before baiting and comprised colony A (Fig. 1). After baits were deployed foragers disappeared from all stations by 7 October 2004, thus eliminating colony A. For 18 mo after baiting, foragers never frequented any of the stations at site 2.

At site 3 three IMs and three MSs were occupied by three colonies (A–C) before baiting and an additional colony (D) after baiting (Fig. 1). Foragers in colony A showed symptoms of benzoyl urea poisoning by 7 October 2004 and were not seen after this date, thus eliminating colony A. Colony C was replaced by colony D by April 2005 (Fig. 1).

At site 4 three IMs and five MSs were occupied by colonies A–D before baiting. Foragers in two IMs and one MS comprised colony A and foragers in the third IM were hydrocarbon phenotype CA-B, not *R. hes-*

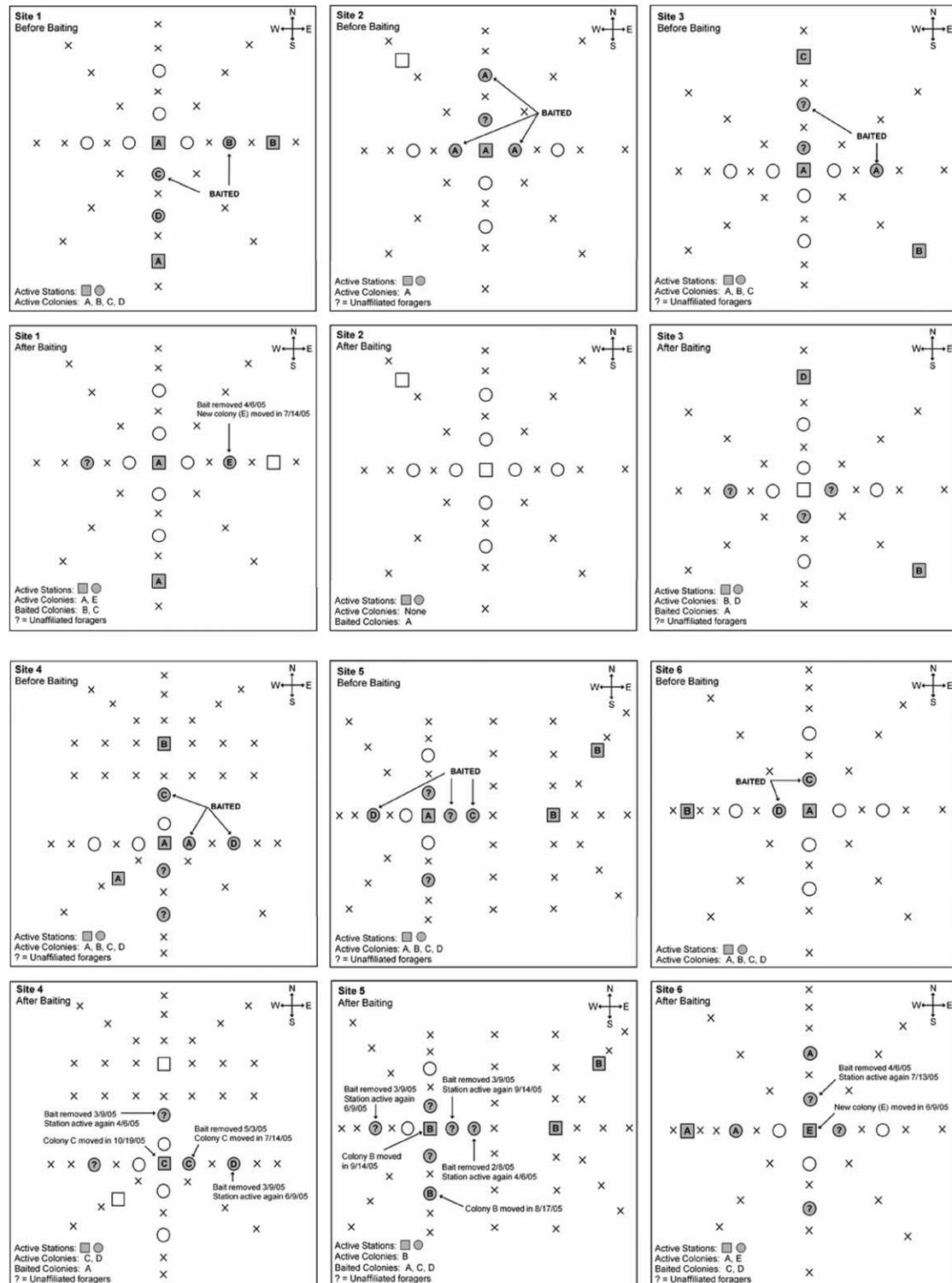


Fig. 1. Schematic placement of Douglas-fir stakes (X) and status of independent monitors (squares) and monitoring stations (circles) at six baited sites in July 2004 (before baiting) and July 2005 (after baiting). Colony designations (A, B, C, D, and/or E) are unique to each site.

perus, and comprised colony B. Two additional colonies, C and D, occupied the other baited MSs (Fig. 1). After baits were deployed foragers in colony A showed symptoms of benzoyl urea poisoning on 10 September 2004 and were never seen at any of the subsequent observations (Fig. 1), thus eliminating colony A. Termites from colony B disappeared after baiting, but we do not know whether they fed on the bait. Colonies C and D resurfaced on 14 July 2005 and 9 March 2005, respectively. Apparently these colonies never fed on the bait, because they did not show signs of benzoyl urea poisoning.

At site 5, three IMs and five MSs were occupied by colonies A–D before baiting. Foragers in the primary IM comprised colony A; the other two IMs were occupied by colony B (Fig. 1). Colonies C and D were found in two of the three baited MSs before baiting (Fig. 1). Foragers in colony A never showed signs of benzoyl urea poisoning but disappeared by 10 September 2004 (Fig. 1). By baiting with lufenuron, it seems that colony A was eliminated, but it is also possible that it abandoned the site. Colony B remained until the close of the study in January 2006. By September 2005, colony B had moved west to occupy all of the IMs, as well as at least one MS (Fig. 1).

Site 6 was a very complex site with numerous colonies, movement of the colonies, and abandonment of IMs or displacement of colonies in an IM by another colony after baiting. Site six had two IMs and two MSs that were occupied before baiting. Foragers in the primary IM comprised colony A and the other IM was occupied by colony B (Fig. 1). Foragers in the two baited MSs comprised colonies C and D (Fig. 1). On 10 September 2004, foragers seen in the primary IM showed signs of benzoyl urea poisoning and were not seen again in this IM from 5 November 2004, until 9 June 2005. Feeding in the primary IM had essentially stopped after 10 August 2004. Colonies C and D vanished, but we have no direct evidence this was a result of consuming bait. Colonies A and B abandoned the IMs that they occupied before baiting. We do not know what happened to colony B. After baiting, colony A moved into the IM 3 m west of the primary IM; colony A remained in this IM until the close of the study in January 2006. By August 2005, colony A had moved into at least two MSs as well (Fig. 1). In June 2005, an additional colony (colony E) moved into the primary IM (Fig. 1) and occupied this IM for only 2 mo, as after July 2005 foragers were not seen again. Colony A was our targeted colony, but it apparently never fed on the baits. The IMs and MSs at site six were visited by five separate colonies, and four colonies (colonies B, C, D, and E) were either eliminated by baiting with lufenuron or abandoned their stations. Because of the uncertainty of the affiliation of foragers in the primary IM in 2005, we chose to not include colony E in assessments of wood consumption (i.e., bait efficacy) or consumption of bait, as doing so might confound our inferences.

Among the six baited sites, six colonies inhabited one or more IM(s) but did not feed on lufenuron bait. Before baiting most of the colonies were limited to one

IM; colony A at sites 1 and 4, and colony B at site 5 occupied two IMs (Fig. 1). Colony territories were often dynamic as individual IMs and MSs were occupied by more than one colony over the span of the study (for example, sites 1, 3–6) (Fig. 1).

Unbaited Sites (7–12). The unbaited sites were established with at least two IMs and the full complement of eight MSs. The MSs were monitored, but baits were never installed. The sites were generally simple with only one IM used.

There were two IMs at site 7; only one was consistently used (Fig. 2). Site 7 had at least one colony that was active in the IM throughout the study. There were two IMs at site 8; only one was used extensively, but only in 2004 (Fig. 2). Only one of the two IMs at site nine was consistently used. Foragers in one of the MSs in site nine were not from the same colony as those in the IM. Thus, site nine had at least two colonies that were active throughout the study (Fig. 2). There were three IMs at site 10, all were consistently occupied, and all of the foragers within belonged to the same colony. Foragers in one of the MSs were not from the same colony as those in the IMs (see Table 2). Thus, site 10 had at least two colonies that were active, but only one colony was consistently active in the three IMs throughout the study (Fig. 2). There were two IMs at site 11. One was only occasionally occupied, mostly in 2004. Five MSs were also visited, but foragers were not confirmed to be associated those in the IM. Thus, site 11 had at least one colony that was active mostly in 2004 (Fig. 2). There were three IMs at site 12. All three were used, but one was abandoned at the end of 2004 (Fig. 2). The colony using the central or primary IM was identified as *R. hesperus*, whereas foragers in the other two IMs were hydrocarbon phenotype CA-B and were members of the same colony. We did not associate the foragers in the MSs with those in any of the IMs. Thus, site 12 had at least two colonies active throughout the study (Fig. 2).

We observed 23 colonies in total at the six baited sites and nine colonies at the six unbaited sites. The colony count could be even greater given foragers in numerous MSs were never documented as being affiliated with an IM or other MSs. Obviously, this arboretum is used by a rich community of *Reticulitermes*, with foraging territories occasionally overlapping or intermingling.

Wood Consumption by Baited and Unbaited Colonies. An important measurement of the success of lufenuron termite bait in controlling colonies was the significant reduction or cessation of wood consumption in the IMs with foragers associated with baited MSs. Termites were found in some of the IMs every month during the study; yet, wood consumption was cyclical and correlated with seasonal weather patterns, similar to a previous study at this location (Haverty et al. 1999b) and in a similar study in central Chile (Ripa et al. 2007) (Fig. 3).

Wood consumption was measured for 18 mo from 3 June 2004 to 15 November 2005. We divided this timeline into: before baiting (3 June 2004–10 August 2004); post baiting I (10 August 2004–5 November 2004);

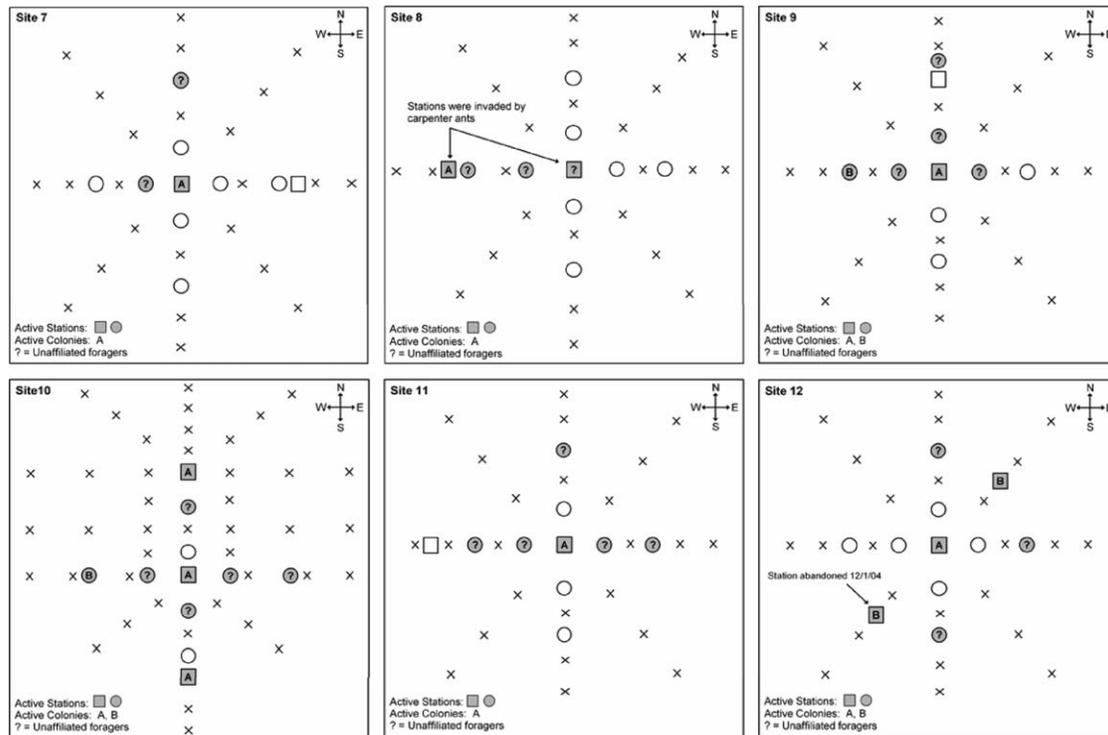


Fig. 2. Status of independent monitors (squares) and monitoring stations (circles) at six unbaited sites throughout 2004 and 2005. Colony designations (A, B, C, D, and/or E) are unique to each site.

doldrums (5 November 2004–3 May 2005); and post baiting II (3 May 2005–15 November 2005) (Table 1). The type 3 tests of fixed effects reveal statistical significance among treatments (baited versus unbaited colonies; $P = 0.0032$), time period (before baiting versus post baiting I versus and post baiting II; $P = 0.0010$), and the interaction of these two factors (treatment and time period; $P = 0.0040$). The covariance parameter estimates resulted in estimates of the serial correlation coefficients of 0.5034 for baited colonies and 0.7002 for unbaited colonies. This is a substantial correlation and suggests that it was appropriate to use repeated measures analysis, as ignoring the serial correlation in time would have resulted in incorrect P values and standard errors.

Wood consumption was significantly different over the three time periods for baited colonies (281.4, 112.5, and 7.9 mg/d, respectively; $P = 0.0017$), whereas the wood consumption was not significantly different for unbaited colonies (590.5, 436.8, and 470.1 mg/d, respectively; $P = 0.2120$) (Table 1). The significant interaction by time period was the result of wood consumption being not significantly different between baited and unbaited colonies for the before baiting (281.4 versus 590.5 mg/d, respectively; $P = 0.4643$) and post baiting I (112.5 versus 436.8 mg/d, respectively; $P = 0.0932$) time periods but significantly different for the post baiting II period (7.9 versus 470.1 mg/d, respectively; $P < 0.0001$) (Table 1). Finally we tested the

difference between the change in mean wood consumption (milligrams per day) from the before baiting time period to the post baiting II time period of baited and unbaited colonies. This was done to determine whether there was an effect caused by baiting. The estimate of this difference in the unitless log-transformed ratios of baited to unbaited is 3.1209 and the P value is 0.0008. This is a highly significant difference that strongly implies that there was a greater change in daily wood consumption in the baited colonies than in the unbaited colonies. This value of 3.1209 is equivalent to a ratio of post baiting II period to before baiting period daily wood consumption ratios of 22.6 [$=\exp(3.1209)$]. Thus, the daily wood consumption ratio for the baited colonies was ≈ 22.6 times as large as the daily wood consumption ratio for the unbaited colonies. In addition, the estimate of the difference in mean wood consumption of the unbaited colonies from the before baiting period to the post baiting II period is not significantly different from zero ($P = 0.6818$). During the 7-mo period, we called the doldrums (5 November 2004–3 May 2005), wood consumption was very low in both the baited colonies (4.3 mg/d) and the unbaited colonies (46.2 mg/d) and was not affected by either treatment. Rather, this low wood consumption was likely the result of the cool soil temperatures that restricted foraging (Haverty et al. 1999b, Ripa et al. 2007).

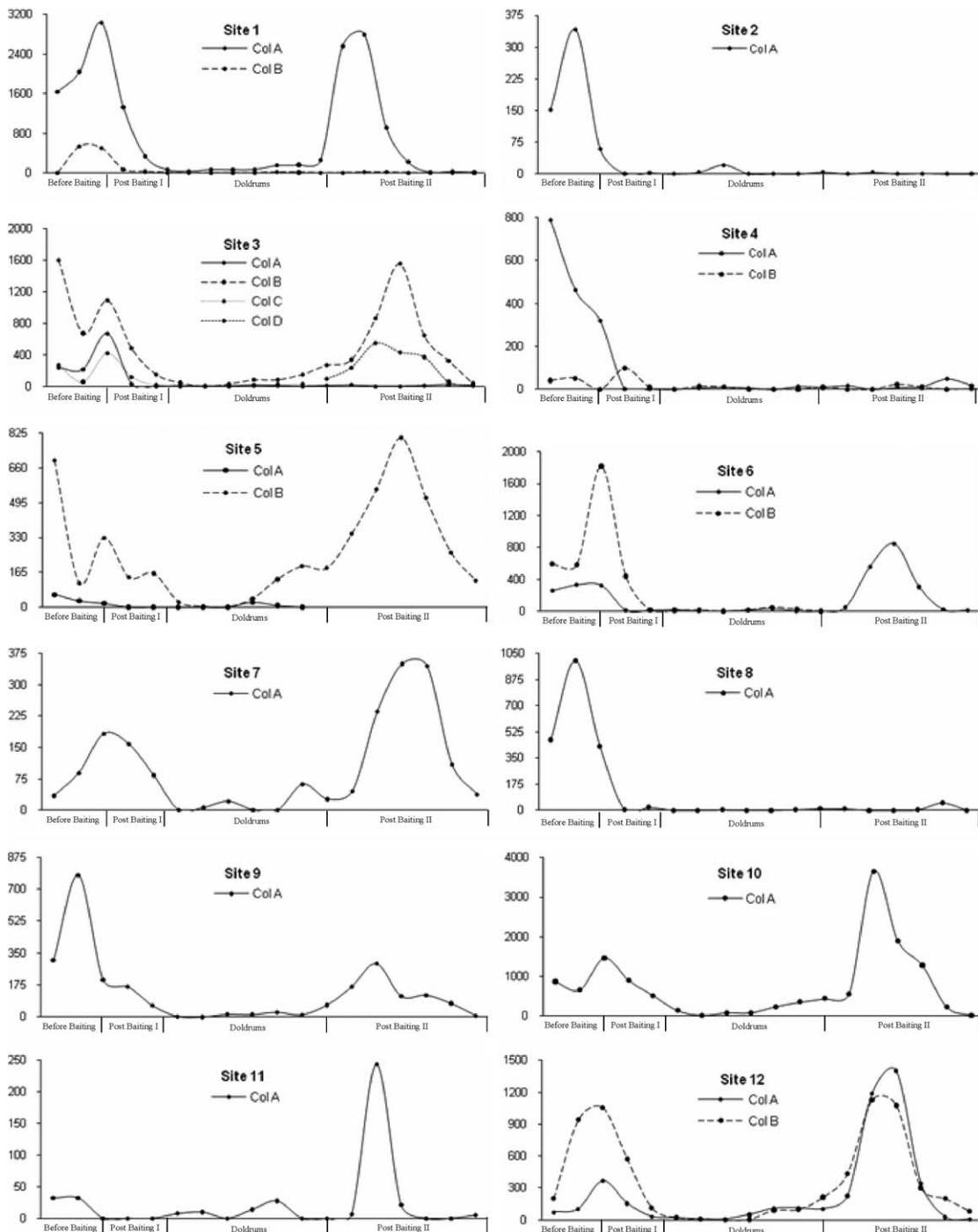


Fig. 3. Wood consumption (milligrams per day) by *Reticulitermes* for each independent monitor at baited (1–6) and unbaited (7–12) sites. Each point represents the mean daily amount of wood consumed (milligrams per day) for that specific month.

The wood lost in the IMs formerly occupied by the baited colonies was significantly greater than zero during the doldrums and post baiting II periods (4.3 ± 1.7 and 7.9 ± 6.6 mg/d, mean \pm 95% confidence interval, respectively), but this is an artifact of the wood-

bundle cleaning technique, because no feeding took place during these times in these IMs. Wooden bundles that were previously fed upon by termites have a much greater surface area than those that were never fed upon by termites. Continual brushing, month after

Table 1. Wood consumption before and after baiting for five baited and 12 unbaited colonies at the Institute of Forest Genetics in Placerville, CA

Site and colony ^a	Wood consumption (mg/d)			
	Before baiting ^b	Post baiting I ^b	Doldrums ^b	Post baiting II ^b
Baited				
1, colony B	265.1	197.4	2.8	5.2
2, colony A	247.0	20.5	4.4	1.6
3, colony A	223.6	231.7	3.2	7.9
4, colony A	626.5	106.9	6.1	16.1
5, colony A ^c	44.9	5.8	5.2	8.5
Mean	281.4a,α	112.5b,α	4.3	7.9c,α
95% Confidence interval ^d	146.2–972.9	40.2–271.2		4.2–33.4
Unbaited				
7, colony A	61.7	141.4	14.5	185.1
8, colony A ^e	736.8	150.1	0.8	11.8
9, colony A	544.3	143.4	11.8	138.7
10, colony A	776.4	965.0	155.4	1,342.3
11 ^f	31.9	0.0	9.6	45.0
16	88.7	185.7	51.6	549.1
Additional unbaited^g				
1, colony A	1,841.2	1,561.9	79.3	1,152.4
3, colony B	1,140.0	575.0	66.6	666.4
3, colony D				290.2
5, colony B	406.2	209.9	65.3	507.5
6, colony A	290.5	114.5	42.0	283.1
6, colony B ^h	588.1	758.0	12.4	
Mean	590.5a,α	436.8a,α	46.2	470.1a,β
95% Confidence interval ^d	456.7–2,842.4	245.6–1,531.0		203.6–1,194.8

Means in the same row, followed by the same letter, are not significantly different ($P \geq 0.05$); means in the same column, followed by the same Greek letter, are not significantly different ($P \geq 0.05$). Values for the doldrums were not tested in the repeated measures analysis of variance.

- ^a See Fig. 3 for site and colony information.
- ^b Before baiting, 3 June–10 August 2004; Post baiting I, 10 August–5 November 2004; Doldrums, 5 November 2004–3 May 2005; Post baiting II, 3 May–15 November 2005.
- ^c A new colony moved into the IM station 14 September 2005. Wood consumption data shown are only for colony A during this period.
- ^d Confidence interval back-transformed from standard deviation generated during repeated measures analysis of variance. Confidence interval for the doldrums time period was not calculated as it was not part of the repeated measures analysis of variance.
- ^e Termites were forced out of the station by carpenter ants.
- ^f Termites abandoned the station during the post baiting I time period.
- ^g These additional unbaited colonies were at baited sites, but did not feed on bait.
- ^h Station changed colonies between 2004 and 2005.

month, resulted in the appearance of wood consumption when there was actually none.

Lufenuron Termite Bait Consumption and Efficacy in Eliminating Colonies of *Reticulitermes*. Bait tubes remained in the MSs for 3 mo to 1 yr. Many were removed because of ant infestations that caused the termites to abandon the bait tubes. Most of the feeding in the bait tubes ceased from 5 November 2004 to 3

May 2005. The mean amount of bait matrix removed by each colony was 8.0 g (≈ 12 mg of active ingredient) with a range of 2.2–16.0 g (Table 2). Within a month of placing the baits in the MSs, the termites found in most bait tubes were opaque, chalky and lethargic compared with unbaited colonies.

It was difficult to assess the amount of time required to eliminate or kill a colony. Wood consumption usu-

Table 2. Number of bait tubes used, amt of bait consumed and days required to eliminate *R. hesperus* colonies at the Institute of Forest Genetics near Placerville, CA

Site and colony ^a	Baited monitoring stations	Bait tubes used	Bait consumed (g)	Total bait consumed (g)	Days from baiting to colony elimination ^b
1, colony B	1-e2	1	9.15	9.15	93 (20 July 2004–21 Oct. 2004)
2, colony A	2-n2	3	10.82	16.0	65 (20 July 2004–23 Sept. 2004)
	2-e1		1.26		
	2-w1		3.91		
	3, colony A	3-e2	1		
4, colony A	4-e1	1	8.33	8.33	65 (20 July 2004–23 Sept. 2004)
5, colony A ^c	5-e1	1	2.18	2.18	37 (20 July 2004–26 Aug. 2004)
Mean				8.03	70.6

- ^a See Figs. 1–3 for site and colony information.
- ^b Based on the absence of termites, cessation of wood consumption in the independent monitor(s) and/or appearance of toxic effects in workers.
- ^c No definitive connection was ever made between the monitoring station and independent monitor for this colony.

ally approached zero between September and October 2004, ≈ 2 –3 mo after baiting (Fig. 3). Confounding this estimate is the seasonal reduction in wood consumption by the unbaited colonies. The end for date for each colony was determined based on a combination of absence of termites, cessation of feeding in the IMs, and apparent toxic effects on foragers. The approximate time required for elimination of a colony ranged from 6 to 12 wk or 37–93 d (Table 2). By June 2005, it was obvious that wood consumption in the baited colonies did not increase (Fig. 3).

Foraging Territories, Movement of Colonies, and Abandonment of Monitoring Stations. Foraging territories were dynamic during the study. Over the course of the study, there were examples a) of foraging territories changing (sites 4, 5, and 6), b) of one colony displacing another (sites 3 and 6), c) of colonies abandoning an IM (sites 3, 4, 6, 8, and 12), d) of a colony moving into vacated territory (sites 1, 3, 4, 5, and 6), and e) of a colony and all termites at a site being eliminated (site 2). Site 6 was exceptional as it exhibited characteristics a–d above and contributed to our inability to understand whether baits impacted any of the colonies at site 6.

Foraging Pressure. Each of the 12 sites seemed to have different foraging pressure as measured in terms of wood consumption (Fig. 3; Table 1), with very high foraging pressure at sites 1, 3, 6, 10, and 12 and very low foraging pressure at sites 2 and 7. The reasons for this involve characteristics that we observed, such as number of colonies/site, and some that we did not observe, such as size of individual colonies and alternative feeding sites. All of these factors could affect assessment of bait efficacy and are reflected in the amount of bait removed and the time to colony elimination or death.

Efficacy of Lufenuron Termite Bait. The main objective of this study was to document the cause and effect of lufenuron termite bait on termite colonies through baiting an active colony and demonstrating that it was killed. Death of a colony can be inferred by either the absence of foragers at baits and IMs, the cessation of wood consumption in the IM occupied by the baited colony, or both. The important issue is to measure termite activity from IMs, not bait stations, that are interconnected with baited MSs so as to avoid measuring repellency of the bait or “learned avoidance” of a toxic bait station (Su and Scheffrahn 1996a). However, to do this it is imperative that we know which colonies fed on the bait and whether these colonies were also frequenting the IMs and when. Because of the complexity of our study location we now recognize that placing bait in more than three MSs may have simplified the results, but not necessarily improved demonstrating performance against a targeted colony. Additionally, the rationale for not baiting all of the active MSs was to minimize the disturbance in the MSs and have alternative MSs to bait, should baited MSs seem to become abandoned without feeding. For operational baiting, one bait station might be sufficient, provided you have a bait station used by each and every colony at a given site. If multiple colonies

are present at a site, foragers from each target colony must access and feed at a bait station for the colony to be eliminated. It is unlikely that foragers from different colonies will feed at the same bait station at the same time due to agonistic behavior (Haverty et al. 1999a, Delphia et al. 2003).

Clearly lufenuron was efficacious in killing *R. hesperus* colonies. Five targeted colonies were eliminated during the course of this study. At one site, site 6, we were unable to determine whether baiting was effective due to insufficient genetic information to understand the dynamics of the colonies at this site. A secondary objective, to determine the amount of time required for colony destruction, was confounded by the reduction in feeding in the fall. However, we estimated that it required 1.5–3 mo for this IGR to be consumed by foragers so that all members of a colony received a lethal dose.

We demonstrated elimination of colonies with the lufenuron termite bait. However, the complexity and dynamic nature of the termite community at this study site, and probably around structures as well, could mask the efficacy of baits. The IMs occupied by one colony that is destroyed as a result of feeding on a bait could be occupied by a different colony after the baits are removed from the MSs. In addition, with the density of colonies at this study site, many colonies in a baited area may never encounter the bait and would escape control.

We were fortunate that we were able to place the baits in the ground on 20 July 2004. Had we waited 1 or 2 mo (until September to October), termites might not have fed sufficiently on the bait until the following spring, possibly further delaying destruction of the colony. Based on information gained in this study, baiting should occur as early as possible in the annual feeding cycle of *R. hesperus*, and possibly other species, targeting bait placement before peak annual wood consumption. Our conclusion is that the MSs should be installed early in the spring and bait deployed early in the feeding cycle, late spring or early summer.

Through the use of genetic analyses, we were able to provide detailed data that linked the deployment of the lufenuron termite bait to the cessation of termite activity of targeted colonies. Equally important, this technique enabled us to avoid falsely stating that lufenuron was ineffective against colonies identified within the baited sites, yet did not feed on the bait. More field studies of this depth are needed to define cause and effect of baits on termite colonies. In addition, newer methods are needed to elucidate underground foraging territories of colonies without having to solely rely on foragers collected at independent monitors and bait stations and techniques used in this study. These studies and techniques would greatly enhance our understanding of termite foraging behavior and territories, resulting in better deployment and effectiveness of termite baits.

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