

Effects of Monitor Examination Intervals on Resource Affinity by *Reticulitermes* spp. (Isoptera: Rhinotermitidae)¹

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J. Entomol. Sci. 46(2): 102-111 (April 2011)

Abstract Monthly visits to 2 field sites in southern Mississippi were made to determine the influence of monitor examination on feeding site affinity by southeastern native subterranean termites (*Reticulitermes* spp.). Wooden board monitors were examined once every 30, 60, or 90 days. Presence of live termites and damage to the boards (both binary parameters) were collected for each plot examined over a 3-yr period, along with monthly soil moisture (as percentage of soil saturation) and temperature at 15.24 cm depth. Time to first occurrence of termite presence and/or damage and subsequent occurrences indicated no difference among the treatments, indicating that none of the treatments influenced termite foraging affinity.

Key Words termite, monitoring rates, foraging affinity, behavior

Lack of activity at termite field sites is often blamed on disturbance, particularly with *Reticulitermes* spp. (Woodrow et al. 2008). Disturbance as a subject in termite literature focuses on two categories of studies: habitat disturbance (Wood et al. 1977, Sarr et al. 1998, Eggleton et al. 1999, Aluko and Husseneder 2007) and individual or small group disturbances (Howse 1965, Stuart 1963, 1988, Kirchner et al. 1994, Hu et al. 2003, Schwinghammer and Houseman 2006). The few field studies that have been published on this subject generally are concerned with the effects of chronic, macroscale environmental changes on termite populations (beyond colony level), such as the importance of agricultural uses on termite abundance/species diversity (Sarr et al. 1998, Eggleton et al. 1999). These papers indicate that cultivated lands are low in termite abundance and diversity, except when the cultivated plants are accepted as food by the termites (Wood et al. 1977, Sarr et al. 1998). These studies were performed in areas of potentially high termite diversity (Africa, Southeast Asia, etc.) where higher populations of termites are common (i.e., Termitidae; many of which are grass, humus, or fungus feeders).

By comparison, North American termites, at least in the continental U.S., are depauperate in terms of species richness (Vargo and Husseneder 2009). Only a few species are present, mainly from the Rhinotermitidae and Kalotermitidae, with few Termopsidae and Termitidae representatives (Kofoid 1934). Localized subterranean

¹Received 09 July 2010; accepted for publication 25 September 2010.

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

termite populations in southeastern forest areas consist mainly of members of *Reticulitermes* Holmgren (Vargo and Husseneder 2009). Environmental changes can influence termite behavior (such as the effects of tree clearing and eventual construction of a home on a lot), and these types of changes can influence termite activity at monitoring or bait stations (Aluko and Husseneder 2007). Small group disturbance studies in laboratories (Hu et al. 2003, Schwinghammer and Houseman 2006, Woodrow et al. 2008, Shelton et al. 2009) are not necessarily applicable to field plot disturbances. A large-scale system needs to be considered.

Because subterranean termites are cryptic animals normally nesting below the soil surface, their behavior and response to certain stimuli are rarely observed directly. Instead, we must infer responses based on indirect evidence of activity, such as actual presence of termites at monitoring stations, or feeding on wood at such stations (Thoms et al. 2009). These difficulties have likely contributed to a paucity of work in this area. However, with current methods of termite control, mainly the use of bait technology, basic information about disturbance and how it relates to termites cannot be ignored.

One type of disturbance is that associated with the examination of wooden monitors. As monitors are examined, termites may leave the feeding site and may or may not return later. The objective of this study was to determine if monitors have a long-term influence on the foraging site affinity of native subterranean termites. The key to providing useful information here is to ensure that the examination is simple enough to be easily, and identically, repeated within and among treatments. In other words, the disturbance regimen should be consistent to prevent variability from influencing the results.

The objective of this study was to determine the effects of simple examination regimen on a population of native subterranean termites (*Reticulitermes* spp.). This study investigated similar examinations of foraged substrates (wooden board monitors) occurring over varying intervals of time.

Materials and Methods

The study examined termite damage to wooden (*Pinus* L. spp.; southern yellow pine) monitoring boards (14 × 14 × 2 cm) placed in the centers of 0.43 × 0.43 m plots (0.19 m²) on 1.52 m (center to center) spacing as a response to examination of the boards on a frequent basis. The plots were placed in a grid within blocks in each of 2 habitats (see below) and randomly assigned a treatment.

Treatments were rates of board examination: once every 30, 60, and 90 d. Due to the differing rates of examination, not all treatments could be sampled simultaneously. To account for this, staggered treatments were included (Table 1) such that by the November 2005 observation (day 90) each treatment type was represented in observations every 30 d.

For installation, debris and leaf litter were removed in plots to expose the bare soil, and a board was placed in the center of the plot. A brick was placed on top of the board to anchor it in place. Each plot was marked with a labeled, painted (by treatment) 0.46 m stake. Stakes were cut from southern yellow pine pressure-treated with Ammoniacal Copper Quat (didecyldimethylammoniumchloride) (ACQ) to prevent termite feeding. Because various treatments would be observed at different times (Table 1), the paint prevented off-schedule movement of boards.

Table 1. Treatment list for monitor examination study of *Reticulitermes spp.* termites on the Harrison Experimental Forest.

Habitat	Treatment	Replicates	Samples	Description
Forest	A	5	3	Examination every 30 days
	B	5	3	Examination every 60 days
	C	5	3	Examination every 60 days
	D	5	3	Examination every 90 days
	E	5	3	Examination every 90 days
	F	5	3	Examination every 90 days
Open	A	5	3	Examination every 30 days
	B	5	3	Examination every 60 days
	C	5	3	Examination every 60 days
	D	5	3	Examination every 90 days
	E	5	3	Examination every 90 days
	F	5	3	Examination every 90 days

The study took place on 2 habitats — a grassy field (open area) and the inner edge of a mixed pine forest (mostly longleaf pine, *Pinus palustris* Miller; forest area) with 5 replicates within each habitat (see below). Each replicate contained 3 plots for each treatment (6 treatments, 3 subsamples each; thus, 18 total plots per replicate, 90 plots per habitat, for a total of 180 plots in the experiment; Table 1).

Plots were established on the Harrison Experimental Forest (HEF, Harrison Co., MS) in August of 2005. HEF is located just north of Gulfport, MS, and whereas unplanned, the plots were installed roughly 1 mo prior to the landfall of Hurricane Katrina. This event occurred shortly before day 30 of this study, but no plots were lost due to the hurricane.

Examinations were made on required plots each month, consequently termite damage and presence of live termites were recorded only for the examined plots at each observation. Additionally, at each observation the soil moisture (as percentage of saturation) and temperature (in °C) at a depth of 15.24 cm were measured using an Aquaterr model T-300 soil moisture/temperature meter (Aquaterr Instruments, Inc.; Costa Mesa, CA) and recorded. Two plastic screw-top opaque buckets (~3.8 L) were filled with water and closed, and one was placed in the center of each habitat (Open 30°38.139'N, 089°02.149'W; Forest 30°38.136'N, 089°02.195'W; ~76.2 m apart) for moisture calibration. Calibrations were made immediately prior to sampling at each habitat (with occasional recalibration during sampling).

To avoid variability, the examination of each monitoring board was kept to a simple, repeatable occurrence. This was done by removing the brick and placing it beside the board. The board was then removed from the substrate in a quick upward motion. The edge of the board was then struck once against the brick to dislodge the soil. At this time, presence of both termite damage and termites was assessed and recorded.

The old board was either replaced with a new board or placed back in the center of the plot according to the conditions below, and the brick placed back on top.

During observations, boards scheduled for examination that showed termite damage (or noticeably infested by fungi) were replaced with new boards. Although it only happened a few times, no data were recorded for missing boards; they were simply replaced. Twice during the study a board was accidentally moved off schedule. In those cases no data were collected, but the board was replaced on the next scheduled observation.

Statistical analysis. Data for both evidence of termite damage on the boards and presence of termites were used in calculating time to first occurrence of termite presence and/or damage for all plots, as well as the number of days between occurrences of termite presence and/or damage for all subsequent occurrences (i.e., first to second, second to third, etc.). Kruskal-Wallis analyses were performed on each habitat separately for the time to first occurrence of termite presence and/or damage, as well as the number of days between occurrences of termite presence and/or damage for the 9 subsequent occurrences ($\alpha = 0.05$; Minitab Inc. 2007).

Presence of termites and their damage data were used to calculate percentages of the plots that had damage (or termites present) for each time period. Separate percentages of plots with evidence of termites and/or their damage were calculated for each treatment as well as each habitat for all time periods starting with day 90 (the first day when all 3 treatments were sampled).

The design does not have a control treatment. If an 'unexamined' control was implemented, it would require placement of boards that would not be examined until the end of the study. It is likely that no boards would survive 3 yrs without replacement even without termite damage, as fungal infestation of boards is common at this site. Monthly examinations of bait stations were somewhat standard until just recently (Thoms et al. 2009). The treatments in this study were compared relative to one another, using the 30-d examination regimen as the standard.

Throughout the experiment, when termites were found they were identified in the field to genus. In late 2008, after the final observation, additional boards were placed in the replicate areas (3 boards per replicate) and examined after 2 months for the presence of termites. Boards containing termites were collected and returned to the laboratory where the termites were extracted and placed in 90% ethanol for identification using the key of Hostettler et al. (1995).

Results and Discussion

The term termite damage in the following paragraphs refers to termite presence and/or damage on the board at the time of examination. Median days to the first 10 occurrences of termite damage are presented in Table 2. Data sets were not normally distributed and required the use of Kruskal-Wallis as opposed to a general linear model (GLM) for analysis. The first occurrence of termite damage occurred in the open habitat after a longer period than in the forested habitat, and the first termite damage occurred in all 3 treatments in an equivalent amount of time in the open habitat. Although the subsequent occurrences of termite damage indicated that there were significant differences among the treatments, these are misleading. The timing of the subsequent termite damage occurrences mimics the minimum number of days between observations for each treatment. In other words, the treatments had no effect from the days to termite damage occurrence standpoint in the open habitat. The forest habitat showed similar results to those from the open habitat on the third to tenth

termite damage occurrences. Interestingly, there was a significant difference in the time to first termite damage occurrence among the treatments, indicating that the first termite damage occurred faster in the 60-d treatment than in the others. From first to third termite damage occurrence, the days to subsequent damage occurrences decreased for all treatments, stabilizing on the third occurrence (Table 2).

Comparing the times to second termite damage occurrence between the habitats shows an interesting effect. Whereas it took longer for the first occurrence of termite damage in the open habitat, there was no gradual decrease as seen in the forested plots (Table 2). This may be due to the lack of alternative food sources in the open habitat, i.e., once found, termites damaged the boards often; whereas, the boards in the forested area where food was more abundant were not damaged as quickly subsequent to the first occurrence of termite damage (Table 2). Data from the forested area showed that the 30 and 60-d treatments took longer for the second occurrence of termite damage than the 90-d treatment. Finally, by the third occurrence of termite feeding, the results were similar to those for the open habitat, indicating a difference among treatments that is easily explained by the fact that these were the minimum numbers of days between observations for those plots. Based on these results, the examination of monitors (at least at these rates) has no effect on *Reticulitermes* spp. foraging affinity.

Percentages of plots with termite damage are illustrated in Fig. 1, separated by habitat and treatment. Based on the results above, one would expect all of these figures to be very similar, if not precisely the same. In all cases percentage of plots with termite damage increased over time with some variability that may be explained by temperature fluctuations throughout the study (Fig. 2). Linear regressions of percentage of plots with termite damage data on observation day are illustrated in Fig. 1 and described in Table 3, and comparisons of slopes within habitat were performed using MTEST with GLM used for intercept comparisons (SAS Institute 1985). For the forested habitat, there were no significant differences [for comparisons of 30 versus 60-d ($F = 0.19$; $df = 1, 35$; $P = 0.665$), 60 versus 90-d ($F = 1.91$; $df = 1, 35$; $P = 0.176$), and

Table 2. Median days to first and 9 subsequent occurrences of evidence of termite feeding in plots in the study.

		Days to:									
		1 st	2 nd	3 rd	4 th	5 th	6 th	7 th	8 th	9 th	10 th
Forest	30	209*	121	34*	31*	33*	34*	34*	35*	33*	28.5*
	60	90*	119.5	62.5*	63*	66*	62*	63*	62*	63.5*	63*
	90	272*	96	92*	96.5*	92*	96*	92*	92*	92*	92*
Open	30	420	34*	31*	30*	31*	31*	33.5*	34*	29*	34*
	60	406	62*	62*	62.5*	66*	62.5*	62*	63*	62*	64*
	90	420	92*	92*	92*	96*	91*	92*	92*	92*	92*

* Indicates a significant difference among treatments for that period, separated by habitat.

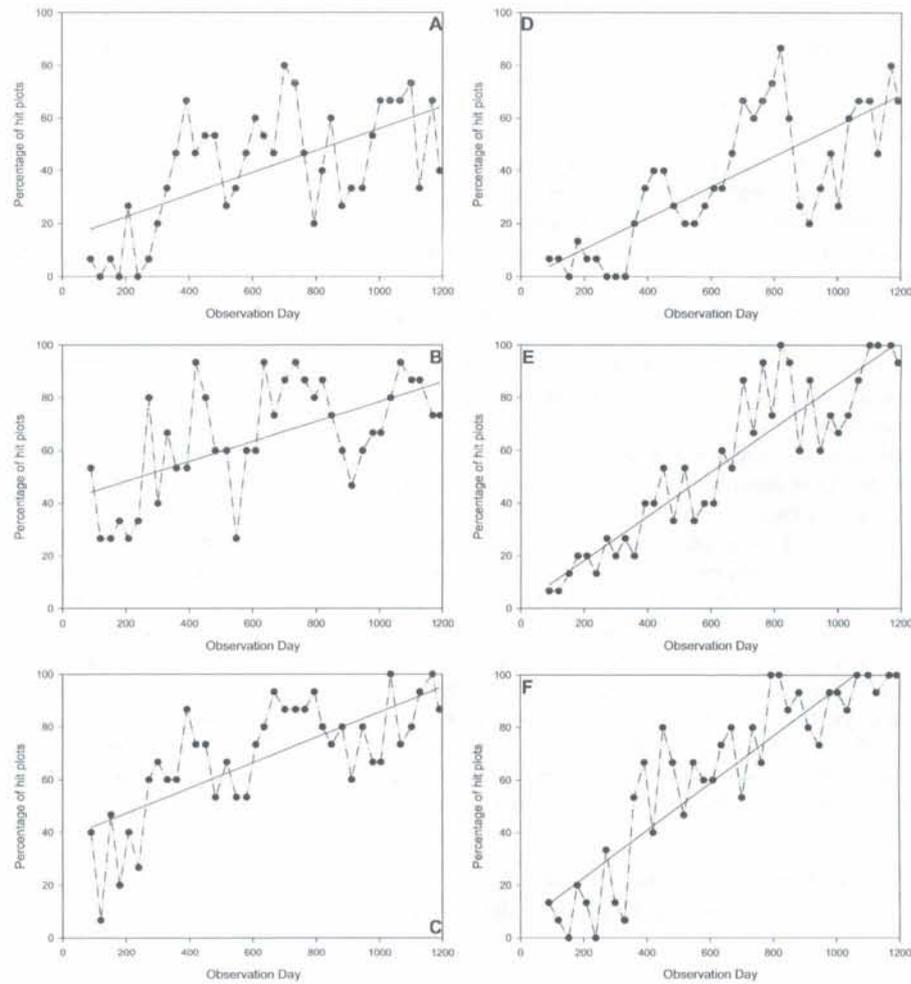


Fig. 1. Percentages of hit plots (those with presence of termites and/or their damage) for each day of observation. A, B and C are forested habitat at 30, 60 and 90-d examination. D, E and F are open habitat at 30, 60 and 90-d examination. Only readings where all 3 treatments are sampled are included (day 90 to end of test). Straight lines represent linear regressions of each graph (details in Table 3).

30 versus 90-d ($F = 0.45$; $df = 1, 35$; $P = 0.505$) among the slopes of all 3 treatments, indicating that they increased at the same rate (Table 3, Fig. 1). Whereas the slopes for the forested habitat were the same, the intercept for the 30-d model was significantly lower than the other 2 treatments ($F = 28.80$; $df = 2$; $P < 0.0001$). For the open habitat, the 30-d model had a significantly different slope from both the 60 and 90-d models [for 30 versus 60-d ($F = 15.25$; $df = 1, 35$; $P = 0.0004$), 60 versus 90-d

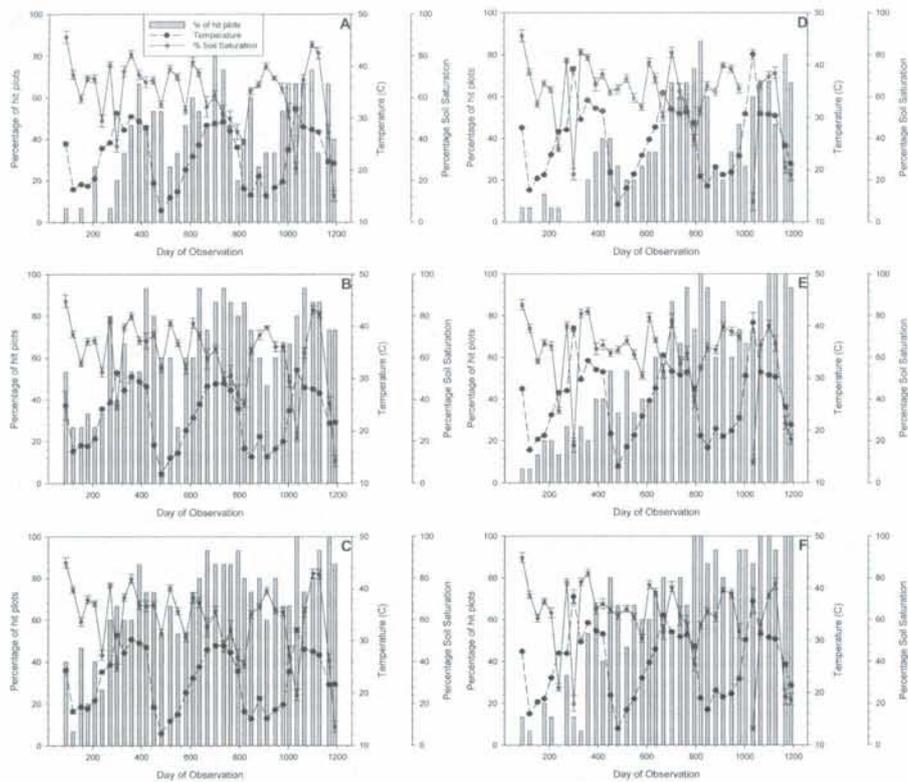


Fig. 2. Mean (\pm SEM) temperature ($^{\circ}$ C) and percentage water saturation of soil at 15.24 cm depth for all plots. Also included are the percentages of hit plots (those with presence of termites and/or their damage) at each day of observation on each graph. A, B and C are forested habitat at 30, 60 and 90-d examination. D, E and F are open habitat at 30, 60 and 90-d examination. Only readings where all 3 treatments are sampled are included (day 90 to end of test). All graphs use the legend given in graph A.

($F = 0.49$; $df = 1, 35$; $P = 0.4904$), and 30 versus 90-d ($F = 13.21$; $df = 1, 35$; $P = 0.0009$) indicating that the 30-d percentages of plots with termites and/or their damage increased less quickly than the 60 and 90-d treatments. It is not surprising that over time more plots had termite damage, as termites discovered boards in each habitat. The two habitats showed differences in the percentage of plots with termite damage. In the forested habitat, only the 90-d treatment was observed to have 100% of plots with termite damage, whereas in the open habitat the 60 and 90-d treatments were observed to have 100% of plots with termite damage by the end of the study (Fig. 1). This may be the result of resource availability in both habitats. The forest habitat had plenty of coarse woody debris for alternate food sources; whereas, in the open habitat food resources were limited to the boards provided by the study.

Table 3. Details of linear regression models describing percentage of plots with termite presence and/or damage over time (Fig. 1).

Habitat	Examination interval	Adjusted r^2	Slope	Intercept
Forest	30	0.35	0.04 (± 0.01)	14.10 (± 6.62)
	60	0.33	0.04 (± 0.01)	40.78 (± 6.25)
	90	0.52	0.05 (± 0.01)	37.55 (± 5.48)
Open	30	0.57	0.06 (± 0.01)	-1.41 (± 5.98)
	60	0.83	0.08 (± 0.01)	1.49 (± 4.56)
	90	0.80	0.09 (± 0.01)	4.73 (± 5.37)

Mean (\pm SEM) temperature values for each collection day are presented in Fig. 2 for both habitats, separated by treatment with the percentage of plots having termite damage included for comparison. The temperature varies annually with lows in December through February, and highs from May to August, with a large increase in June of each year (Fig. 2). For both habitats and all treatments, percentage of plots having termite damage throughout the last 2 yrs of the study cycles with temperature in Fig. 2, with fewer plots having termite damage during the colder months and more plots with termite damage during the warmer months. Figure 2 also illustrates the mean (\pm SEM) values for soil percentage saturation at each collection date.

As a means of determining if all plots among treatments were equivalent, the temperature and moisture data were subjected to GLM in SAS (SAS Institute 1985), comparing habitat, day of observation, treatment, and all possible interactions. Only habitat ($F = 8,967.82$; $df = 1$; $P < 0.0001$), day ($F = 3,897.29$; $df = 36$; $P < 0.0001$), and their interaction ($F = 77.66$; $df = 35$; $P < 0.0001$) significantly influenced soil temperature in this study, indicating that plots were similar across treatments. The results of the moisture data GLM were similar to those for temperature in that habitat ($F = 15.75$; $df = 1$; $P < 0.0001$), collection date ($F = 258.27$; $df = 36$; $P < 0.0001$), and their interaction ($F = 16.35$; $df = 35$; $P < 0.0001$) significantly influenced soil moisture. Treatment also significantly influenced soil moisture in these data ($F = 5.20$; $df = 2$; $P = 0.0056$). However, the difference in mean soil saturation between the treatments/habitats with the greatest (30-d disturbance/open habitat; $62.15 \pm 0.79\%$, $n = 518$) and the least (90-d disturbance/forest habitat; $60.32 \pm 0.81\%$, $n = 554$) mean soil moisture was less than 2% and unlikely to be biologically important. The habitat differences in temperature and moisture were expected due to seasonal variation and the cover provided by the forest canopy.

During the study the field identification of termites always resulted in *Reticulitermes* spp. Of the 30 boards set out for collecting termites after the study, only half (15) had evidence of termite damage upon collection. Due to the low numbers of boards having live termites, additional collections were made from other pieces of coarse woody debris (4 additional collections) within the replicate areas. From all the collections, only 10 contained soldiers. Of those, 6 were identified as *R. virginicus* Banks and 4 were *R. flavipes* (Kollar). Both habitats contained individuals from each species.

Studies of disturbance in laboratory trials have indicated that termites will eventually return to disturbed sites (Schwinghammer and Houseman 2006, Woodrow et al. 2008, Shelton et al. 2009). In those experiments termites are confined and the level of confinement varied among studies. Most of the studies provide a means for termites to escape and avoid the disturbances, whereas in the current study termites are completely unconfined and the level of threat is small. Our results indicate that termites will readily return to feeding sites after monitors are examined, but are inconclusive in determining the amount of time necessary between examinations to affect termite foraging affinity. It appears to be less than 30 days (Table 2). In fact, with such large intervals, it is entirely possible for termites to move into and out of monitors numerous times between readings.

Acknowledgments

The authors thank Craig D. Bell, Nathan T. Brown, Shawn M. Cooper, Donald I. Fye, and E. Lee Scruggs for their technical assistance during this study.

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