Response of Subterranean Termites (Isoptera: Rhinotermitidae) to Stressed Nestmates[1]

T.G. Shelton[2], J.K. Grace[3], R.J. Woodrow[4], and R.J. Oshiro[3]

U.S. Department of Agriculture Forest Service; Southern Research Station; Insects, Diseases, and Invasive Plants; 201 Lincoln Green, Starkville, Mississippi 39759 USA

J. Entomol. Sci. 46(4): 284-291 (October 2011)

Abstract *Reticulitermes flavipes* (Kollar), *R. virginicus* Banks, and *Coptotermes formosanus* Shiraki were stressed by holding them at 25°C or 28°C, respectively, in metal pans for 5 d prior to introduction into 1 side of 3-jar arenas (3 containers connected serially) containing freshly-collected nestmates. After 14 d, mass loss of wooden blocks was determined for blocks from the arena side to which stressed termites were added (treated) as well as those from the opposite side (control). Feeding effects of termite exposure to stress were inconsistent among species. For *R. flavipes*, there was no significant difference in feeding; whereas loss of block mass on the treated side in *R. virginicus* arenas was significantly greater ($P = 0.0017$) than that on the control side of the arenas. For *C. formosanus,* mass loss of wafers also was significantly greater ($P = 0.0284$) on the treated side of the arenas, similar to the response of *R. virginicus*.

Key Words disturbance, stress, feeding, termite, *Reticulitermes, Coptotermes*

Native subterranean termites belonging to the genus *Reticulitermes* Holmgren are widespread throughout the southeastern U.S. (Kofoid 1934). Whereas beneficial in their natural forest habitat, these insects become pests by feeding on structures (Kofoid 1934). The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is a widespread invasive pest of structures found in many southeastern states, as well as Hawaii and southern California (Woodson et al. 2001). Various methods are used in termite management, including wood preservatives and termite-resistant building materials. Efficacy testing for both of these methods of control often involves multiple choice tests, in which a group of termites in the laboratory is presented with several different food choices. Termites are selective in their feeding, often choosing a single wooden block of several apparently identical blocks on which to feed first (LaFage and Delaplane 1987). Feeding choice tests are used to determine which treatments are most repellent among a variety of choices, with inconsistent choices made by the termites interpreted as indicating equivalence among the materials available.

[1]Received 18 February 2011; accepted for publication 11 April 2011. 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.

[2]Address inquiries (email: tshelton@fs.fed.us).

[3]College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, Honolulu HI 96822.

[4]Center for Life and Health Sciences, Mohawk Valley Community College, 1101 Sherman Drive, Utica NY 13501.

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-07-05 via free access

Studies of wood preservatives often require laboratory efficacy trials using wooden blocks to determine the amount of mass loss attributed to termite feeding (e.g., Grace and Yamamoto 1992, Grace et al. 1993a, b). Many factors other than the preservative treatment can potentially influence termite choice in such tests (Waller 1988), for example, growth-ring numbers and block cut orientation (Lindsey 2010), size of block (Howick 1975, Lenz 2005), and wood species (Smythe and Carter 1970a, b). Correcting for these influences is desirable, because it can result in more consistent evaluations of termite feeding on test materials.

Disturbance of termites during laboratory tests also can influence the results. Addition of dead nestmates to an arena where termites are feeding can lead to a reduction in feeding on blocks spatially associated with the dead nestmates (Woodrow et al. 2008, Wong and Lee 2010). Whereas it is not normal for large numbers of dead termites to be deliberately added to a feeding test, it is likely that termites that have been maintained for brief periods under less than optimal conditions may indeed be used in such a study (e.g., LaFage and Delaplane 1987). Thus, the influence of these stressed termites on the results of feeding tests needs to be established.

It is possible that short-term storage, such as keeping termites separate from their host wood in an incubator for several hours, days, or weeks, may impact feeding tests. Because termites are generally collected from the field several days, or even several weeks, prior to use in the laboratory, such disturbance via storage is a likely scenario. The following study evaluates the feeding response of freshly-collected termites when encountering stressed nestmates. Whereas there are many conditions that may stress termites (i.e., suboptimal temperature, humidity, etc.) and termite responses to each may vary, removal from host wood was used as the stressor in this study. In the present study, we examined the feeding response of groups of *Reticulitermes flavipes* (Kollar), *R. virginicus* Banks, and *C. formosanus* confronted with nestmates that had been kept under stressful conditions. The effects of introducing stressed termites into feeding tests were examined by holding a population of termites in artificial conditions (away from their host wood) for 5 d before adding them to unstressed nestmates in an in-progress feeding choice test.

Our null hypothesis was that addition of stressed nestmates would have no influence on feeding on either wood block in this two-choice assay, and that block mass loss would not demonstrate any differentiation by the termites among replicates. If the null hypothesis was true, the termites within an arena were expected to cluster at one food source or the other (LaFage and Delaplane 1987, Peterson et al. 2004). However, across all the arenas it is expected that termites in an even number of arenas would cluster on each alternative; therefore, feeding on each block should be roughly equal if the null hypothesis was true. The alternative hypothesis was that a difference would exist between feeding upon wood blocks closer to the point of addition of the stressed individuals to the feeding arena, and those blocks placed further from this initiation site.

Methods and Materials

Reticulitermes spp. Termite-infested downed timber was located in the John W. Starr Forest maintained by Mississippi State University, in Starkville, MS. Termites were collected by transporting <1 m lengths of the infested lumber to the laboratory and storing them in 114 L metal trash cans until needed for experiments. Termites were identified as *R. flavipes* or *R. virginicus* using soldier morphology as described

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-07-05 via free access

by Hostettler et al. (1995). Methods and materials for both species were similar, with a few exceptions noted below.

On Day 1 of the study, ~1000 termites (natural caste mix) were extracted from the infested lumber and placed into a metal chafing dish (30.48 × 25.4 × 6.35 cm) and provided with 10 Whatman #2 (9 cm diam; Whatman International Ltd., Maidstone United Kingdom) filter papers moistened with deionized water (~1 ml per filter paper). The lid was replaced on the chafing dish, and the covered dish was placed into an incubator maintained at 25 ± 1°C and >70% R.H. until Day 5 of the study. Deionized water was added daily to remoisten the filter papers. Prior to the initiation of the study, 10 arenas were constructed consisting of 3 8-cm diam × 10 cm tall screw top plastic jars (473 ml, Qorpak, Bridgeville, PA), connected using 4 cm lengths of flexible plastic tubing (~1.2 cm outer diameter) through holes near the bottom of each jar, and sealed in place with silicone caulk (GE Silicone II, 100% Silicone window and door sealant, General Electric, Huntersville, NC) so that a 2 cm distance was kept between adjacent jars. Jars were connected in series such that for each arena there were 2 outer jars and 1 center jar identical to arenas in Woodrow et al. (2008).

On Day 2 the jars were filled with 150 g of silica sand (Thermo Fisher Scientific, Waltham, MA), with plastic drinking straws in place to create tunnels from each tube ending to the sand surface in all jars. This was accomplished by placing one end of the straw into the opening of the plastic tubing before adding sand and water to the jar. Deionized water (27 ml) was added to each jar, and the straws were removed. Finally, a southern yellow pine (*Pinus* L. spp.) block (1.27 × 1.27 × 1.27 cm) was added to each outer jar. Blocks were drawn from the same lot as the blocks below, but were not autoclaved, dried or weighed prior to use. All arenas were taped to fiberglass boards (20.32 × 40.64 cm) before placing in an incubator at 25 ± 1°C and >70% R.H. Additionally, on Day 2 of the study, 20 1.27 × 1.27 × 1.27 cm southern yellow pine (*Pinus* spp. L.) blocks were labeled in pencil, autoclaved (dry setting, 45 min), dried at 90°C for 24 h, cooled for 1 h in a desiccator containing Drierite® (W.A. Hammond Drierite Company Ltd., Xenia, OH; ~1 - 3% R.H.), and masses were determined. The blocks were set aside until Day 5 of the study.

On the third day of the study, ~2500 termites were collected from the same colony source as on Day 1. These termites were counted into groups of 200, consisting of 195 workers (pseudergates of the third instar or older) and 5 soldiers for *R. flavipes*. With *R. virginicus*, few soldiers were available, and 200 workers were used in each replicate. These groups were added to the center jars of the arenas described above, lids closed loosely and each arena was returned to the incubator.

On Day 5, termites from the chafing dish were counted into groups of 40 termites (39 workers and 1 soldier; a 20% increase in termites in the arenas). With *R. virginicus,* only workers were used in this group as well. These termites held for 5 d before use and were considered to be "stressed" termites. The wooden blocks were removed from both outer jars in each arena and discarded. For each arena, a coin toss determined which of the 2 outer jars would receive the stressed termites. Five minutes after addition of the stressed termites, the preweighed blocks described above were placed within the outer jars. All jar lids were loosely closed, and the arenas were returned to the incubator.

Arenas remained in the incubators for 14 d. Every other day, the incubator was opened briefly and the termite activity within each jar estimated using a scale of 0 - 5 with 0 = 0%, 1 = 1 - 20%, 2 = 21 - 40%, 3 = 41 - 60%, 4 = 61 - 80%, and 5 = 81 - 100% of the total number of termites present in the arena. On the final day, termite activity

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-07-05 via free access

was again assessed before breakdown of the arenas. For breakdown, each jar within each arena was removed and the surviving termites counted separately. Blocks were placed in marked Petri dishes and cleaned of sand and termite feces, then dried, cooled and reweighed as described above.

Percentage block mass loss was subjected to paired *t*-tests in SAS (SAS Institute 1985). Greater than 25% of the termites escaped during the test from 4 of the *R. virginicus* replicates, and these were removed from analysis. Additionally, 1 block from the *R. virginicus* trial was damaged whereas cleaning termite feces, and that replicate was also dropped from the analysis.

Coptotermes formosanus. Methods for *C. formosanus* generally followed those given above for *Reticulitermes* spp. with the following exceptions. Arenas were constructed using Amazing GOOP household adhesive (Eclectic Products, Inc., Pineville, LA) instead of silicone glue, and no aluminum tape was used. The number of termites added to the jars remained the same at 200. However, because *C. formosanus* colonies have higher soldier complements than *Reticulitermes* (Haverty 1977, 1979), the initial number of termites added to each arena was 180 workers and 20 soldiers (10% soldiers). On Day 5, 40 stressed termites (36 workers and 4 soldiers) were added to each arena. Arenas were taped to 30.48 cm × 30.48 cm metal sheets for support. Southern yellow pine wafers (2.54 × 2.54 × 0.635 cm) were used instead of the 1.27 cm cubes used in the *Reticulitermes* trials. Choice tests with *C. formosanus* in the literature often use a temperature of 28°C (e.g., Grace et al. 1996, Cornelius et al. 1997); therefore, this temperature was used in the current study. Arenas were kept in incubators at 28 ± 1°C, and observations were made twice weekly as described above.

Results and Discussion

Mean (±SEM) percentage mortality at the end of 14 d for *R. flavipes* was 18.28 ± 1.25%. This value includes both mortality and the loss of escaped termites. As noted above, 4 replicates of *R. virginicus* were removed due to termites escaping; percentage mortality in the remaining 6 replicates was 14.10 ± 0.99%. For *C. formosanus*, percentage mortality was 6.71 ± 0.82%.

Tables 1 - 3 describe the visible activity of each species (*R. flavipes* in Table 1, *R. virginicus* in Table 2, *C. formosanus* in Table 3) over time, in both the treated jars (where stressed termites were released) and control jars. The tables provide the percentage of jars (either treated or untreated) that fall into each activity category (0 - 5). For *R. flavipes*, the activity data appeared equivalent between the treated and the control jars, with only a slight increase in the values for category 0 (no termites active) in the treated jars starting on Day 7 (Table 1). For *R. virginicus*, the activity data indicate that there were more termites present in the treated jars than in the untreated jars throughout the study (Table 2). For *C. formosanus*, no signs of repellency are present in the activity data, and these termites also did not show a clear preference for either the treated or the untreated jars (Table 3).

At the end of each trial the surviving termites remaining in each jar were counted. The percentage of termites found in the treated jars was determined as a function of the total surviving termites in each arena. None of the 3 data sets were normally distributed. Median percent of termites found in the treated jars for *R. flavipes* was 22.1% (IQR = 2.1, 78.1), 31.5% (0.4, 77.9) for *R. virginicus*, and 42.8% (20.1, 89.0) for *C. formosanus*. As can be seen from the interquartile ranges, the percentages of

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-07-05 via free access

Table 1. Percentage of jars falling into each *R. flavipes* activity category (0 - 5) over time (day of study)*.

	Treated Side						Untreated Side					
Day	0	1	2	3	4	5	0	1	2	3	4	5
7	40%	40%	20%	0	0	0	50%	10%	40%	0	0	0
9	44%	56%	0	0	0	0	56%	44%	0	0	0	0
12	56%	22%	22%	0	0	0	44%	44%	11%	0	0	0
13	56%	22%	22%	0	0	0	33%	33%	33%	0	0	0
15	56%	33%	11%	0	0	0	44%	33%	22%	0	0	0
17	56%	44%	0	0	0	0	33%	44%	22%	0	0	0
19	67%	33%	0	0	0	0	33%	44%	22%	0	0	0

*Note: One replicate was removed on the second reading due to termite escapes from that arena.

termites remaining in the treated jars varied considerably. These values indicate that, overall, fewer than half of the termites remained in the treated jars at the end of the study. Only the value for *C. formosanus* approaches the even distribution described in the activity data. These values contradict the activity data for both *Reticulitermes* spp. Termites exhibit a range of behaviors (tunneling, building, etc.) in artificial environments (Whitman and Forschler 2007), and it should be noted that presence of termites in a jar during the activity data collection did not necessarily indicate feeding.

Figure 1 illustrates the percentage mean block mass loss for all 3 species. For *R. flavipes*, percentage mean mass loss of the blocks on the control side of the arenas (the side that did not have stressed termites added) was 16.73 ± 2.14%. Percentage mean mass loss of the blocks on the treated side (the side that had stressed termites

Table 2. Percentage of jars falling into each *R. virginicus* activity category (0 - 5) over time (day of study)*.

	Treated Side						Untreated Side					
Day	0	1	2	3	4	5	0	1	2	3	4	5
7	0	0	17%	83%	0	0	67%	17%	17%	0	0	0
9	0	17%	17%	67%	0	0	17%	67%	17%	0	0	0
11	0	17%	17%	67%	0	0	83%	0	17%	0	0	0
13	0	0	50%	50%	0	0	33%	67%	0	0	0	0
15	0	0	67%	33%	0	0	50%	50%	0	0	0	0
17	0	17%	17%	67%	0	0	50%	33%	17%	0	0	0
19	0	0	67%	33%	0	0	17%	67%	17%	0	0	0

*Note: Four replicates were removed due to excessive termite escaping.

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-07-05 via free access

Table 3. Percentage of jars falling into each *C. formosanus* activity category (0 - 5) over time (day of study).

	Treated Side						Untreated Side					
Day	0	1	2	3	4	5	0	1	2	3	4	5
5	0	70%	30%	0	0	0	0	90%	10%	0	0	0
9	0	20%	40%	40%	0	0	0	10%	70%	10%	0	10%
11	0	10%	50%	30%	10%	0	0	50%	30%	20%	0	0
16	0	10%	40%	40%	10%	0	0	50%	30%	20%	0	0
18	0	10%	50%	30%	10%	0	0	40%	40%	20%	0	0
19	0	10%	40%	30%	20%	0	0	30%	50%	20%	0	0

added) was 11.69 ± 2.29%. Differences between the 2 groups were tested using a paired *t*-test ($t = -2.21$; df = 7; $P = 0.0625$) which indicated that the presence of stressed nestmates did not influence *R. flavipes* feeding. This is consistent with the activity data for *R. flavipes* which did not indicate a preference between the blocks (Table 1).

Reticulitermes virginicus did not respond to the inclusion of stressed nestmates in the same manner as *R. flavipes* in this multiple choice test. Blocks in the treated and

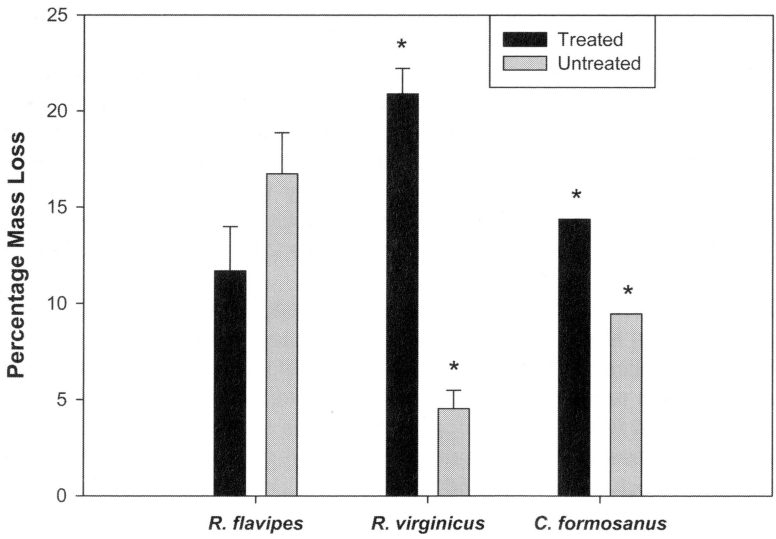

Fig. 1. Mean (± SEM; median for *C. formosanus*) percentage mass loss of blocks in the treated side (side where stressed termites were released) and the untreated side of arenas, separated by species. Asterisks indicate significance at the 0.05 level (paired *t*-tests, Kruskal-Wallis for *C. formosanus*) between groups within species.

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-07-05 via free access

untreated sides of the arenas differed significantly in mass loss (t = 7.52; df = 4; P = 0.0017). Blocks removed from the treated side of the arenas had mean mass losses of 20.89 ± 1.33%, whereas those removed from the untreated sides of the arenas had mean mass losses of 4.53 ± 0.958% (Fig. 1). This, and the activity results presented previously, indicate that the *R. virginicus* groups were not adversely influenced by the addition of stressed nestmates and that the disturbance may have actually stimulated feeding in the immediate vicinity (Table 2).

Coptotermes formosanus responded to the stressed individuals in a manner similar to *R. virginicus*. Median percentage wafer mass loss for the control wafers was 9.47 (IQR = 6.92, 13.95), and for the treated (stressed side) wafers was 14.38 (10.27, 17.91) (Fig. 1). Percentage mass loss data were not normally distributed; therefore, the hypothesis was tested using Kruskal-Wallis analysis (Proc Npar1way; SAS Institute 1985). Wafers removed from the treated side of the arenas had significantly greater percentage mass loss (X^2 = 4.8057; df = 1; P = 0.0284) than those removed from the control side of the arenas (Fig. 1). This indicates that, as with *R. virginicus*, the addition of stressed individuals to the arenas may have stimulated feeding in the treated jar. However, this contradicts the relatively even distribution of termites observed in the activity data (Table 3).

The 2 *Reticulitermes* species differed in their response to the presence of stressed nestmates. In both species, there was no repellency associated with the stressed termites. In fact, block choice by *R. virginicus* appeared to be positively correlated with the introduction point of the stressed individuals. This raises the question of whether the presence of stressed individuals may help to recruit nestmates to nearby food resources (LaFage and Delaplane 1987). We speculate that for *R. virginicus* and *C. formosanus*, the presence of stressed individuals represents a difficult foraging situation (for example broken or missing tunnels outside of soil) which leads to increased recruitment to that area. This may be adaptive, as increases in worker numbers could repair damaged tunnels in the previous example. It would be interesting to see what effect, if any, stressed individuals may have on recruitment of soldiers to an area. Alternatively, it may be that there is no recruitment of additional workers, and the differences in feeding on these blocks was due simply to the additional foragers (the stressed termites) added to the arena. However, such a large difference (particularly for *R. virginicus*; Fig. 1) would be unlikely from only a 20% increase in workers on one side.

Our results demonstrate that different termite species may respond differently to the same nonlethal stress factor. Our study attempted to replicate an extreme scenario in which termites might be held several days before their use in a laboratory test. However, there are numerous possible stress factors, each of which may induce different responses in termites. We suggest that future studies of this phenomenon include behavioral observations of healthy termites interacting with stressed individuals to determine what changes in individual interactions may be associated with the differences in food choice, recruitment and feeding.

Acknowledgments

The authors thank Craig D. Bell for expert technical assistance during these studies. Research at the University of Hawaii was partially supported by USDA-ARS Specific Cooperative Agreements 58-6435-8-294 and 58-6435-0-496, and McIntire-Stennis funds managed by the College of Tropical Agriculture and Human Resources.

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-07-05 via free access

References Cited

Cornelius, M. L., J. K. Grace and J. R. Yates. 1997. Toxicity of monoterpenoids and other natural products to the Formosan subterranean termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 90: 320-325.

Grace, J. K. and R. T. Yamamoto. 1992. Termiticidal effects of a glycol borate wood surface treatment. For. Prod. Journ. 42: 46-48.

Grace, J. K., P. E. Laks and R. T. Yamamoto. 1993a. Efficacy of chlorothalonil as a wood preservative against the Formosan subterranean termite. For. Prod. Journ. 43: 21-24.

Grace, J. K., R. T. Yamamoto and P. E. Laks. 1993b. Evaluation of termite resistance of wood pressure treated with copper napthenate. For. Prod. Journ. 43: 72-76.

Grace, J. K., D. M. Ewart and C. H. M. Tome. 1996. Termite resistance of wood species grown in Hawaii. For. Prod. Journ. 46: 57-60.

Haverty, M. I. 1977. The proportion of soldiers in termite colonies: a list and a bibliography. Sociobiol. 2: 199-216.

Haverty, M. I. 1979. Soldier production and maintenance of soldier proportion in laboratory experimental groups of *Coptotermes formosanus* Shiraki. Ins. Soc. 26: 69-84.

Howick, C.D. 1975. Influences of specimen size, test period and matrix on the amounts of wood eaten by similar groups of laboratory termites. Record of the 25th British Wood Protection Association Ann. Conv. Pgs 51-58.

Hostettler, N. C., D. W. Hall and R. H. Scheffrahn. 1995. Intracolony morphometric variation and labral shape in Florida *Reticulitermes* (Isoptera: Rhinotermitidae) soldiers: significance for identification. Fla. Entomol. 78: 119-129.

Kofoid, C. A. 1934. Termites and termite control. Univ. California Press, Berkeley.

LaFage, J. P. and K. S. Delaplane. 1987. Variance in feeding on equivalent wood blocks by the Formosan subterranean termite (*Coptotermes formosanus* Shiraki). Proc. Intern. Research Group on Wood Protection. Document 1325: 1-7.

Lenz, M. 2005. Laboratory bioassays with termites - the importance of termite biology. Proc. Intern. Research Group on Wood Protection. IRG/WP 05-10550.

Lindsey, G. B. 2010. Evaluation of parameters specified in AWPA standard E1-97 "Standard method for laboratory evaluation to determine resistance to subterranean termites." M.S. Thesis. Mississippi State Univ.

Peterson, C. J., P. D. Gerard and J. Ems-Wilson. 2004. Recommendations for treated-area choice assays with termites (Isoptera). Sociobiol. 44: 171-185.

SAS Institute. 1985. SAS user's guide: statistics. SAS Institute, Inc., Cary, NC.

Smythe, R. V. and F. L. Carter. 1970a. Feeding responses to sound wood by *Coptotermes formosanus, Reticulitermes flavipes*, and *R. virginicus* (Isoptera: Rhinotermitidae). Ann. Entomol. Soc. Am. 63: 841-847.

Smythe, R. V. and F. L. Carter. 1970b. Survival and behavior of three subterranean termite species in sawdust of eleven wood species. Ann. Entomol. Soc. Am. 63: 847-850.

Waller, D. A. 1988. Host selection in subterranean termites: factors affecting choice. Sociobiol. 14: 5-13.

Whitman, J. G. and B. T. Forschler. 2007. Observational notes on short-lived and infrequent behaviors displayed by *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). Ann. Entomol. Soc. Am. 100: 763-771.

Wong, N. and C.-Y. Lee. 2010. Effects of disturbance and the presence of termite and other invertebrate carcasses at feeding sites on the behavior of the subterranean termite *Microcerotermes crassus* (Blattodea: Termitidae). Sociobiol. 55: 353-367.

Woodson, W. D., B. A. Wiltz and A. R. Lax. 2001. Current distribution of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in the United States. Sociobiol. 37: 661-671.

Woodrow, R. J., T. G. Shelton, R. J. Oshiro, J. K. Grace and T. L. Wagner. 2008. Effects of disturbance-related trauma on foraging by subterranean termites (Isoptera: Rhinotermitidae). Sociobiol. 52: 107-118.

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-07-05 via free access