Lack of Preference by *Reticulitermes* spp. (Isoptera: Rhinotermitidae) for Termite Feeding Stations With Previous Termite Exposure[1]

Janine M. Polizzi and Brian T. Forschler

Department of Entomology, University of Georgia, Athens, Georgia 30602 USA

J. Agric. Urban Entomol. 16(3): 197–205 (July 1999)

ABSTRACT Baiting control strategies for population management of subterranean termites requires feeding at either detection, monitoring, or bait stations. We tested the hypothesis that previous inter- or intraspecific termite feeding on cardboard baits would not influence subterranean termite feeding at a station. Feeding sites, consisting of 50-ml plastic-lidded centrifuge tubes containing corrugated cardboard with and without previous termite exposure were tested for acceptance by worker termites from three populations of *Reticulitermes flavipes* (Kollar) or *Reticulitermes virginicus* Banks in a choice feeding assay. Termites were given a choice of four feeding stations that were either previously exposed to nestmates, conspecifics, allospecifics, or that had no previous termite contact. Differences in corrugated cardboard consumption rates among treatments were used as a measure of feeding-site preference. There were no statistically significant differences in mean corrugated cardboard consumption rates among treatments for either species. Yet, these studies indicate the need to more closely examine the feeding site selection process in subterranean termites.

KEY WORDS subterranean termite, feeding, cardboard consumption, bait

Termite baiting strategies are a recent addition to the arsenal of control tactics available against subterranean termites (Potter 1997). Termite baiting strategies use devices intended to detect termite activity by presenting a suitable bait matrix to a termite population (Potter 1997). With the possibility of termite populations moving into and out of an area (Su & Scheffhran 1988, Forschler 1996), the question arises, Can termite detection, monitoring, or bait stations be reused if they were in previous contact with other termites? A subterranean termite population feeding at a bait site may be removed either by elimination or avoidance (Forschler 1996, Su & Scheffhran 1996) and another population could then move to that site because it still provides a palatable feeding substrate – the detection, monitoring, or bait station (Forschler 1996).

Delaplane & La Fage (1989a) reported that *Coptotermes formosanus* Shiraki preferred wood previously damaged by conspecifics over wood damaged by *Reticulitermes virginicus* Banks and also preferred *R. virginicus*-damaged wood over virgin wood. Delaplane & La Fage (1989a) attribute these preferences to thigmotactic cues based on a lack of termite response to an assay of hexane-soluble extracts from the wood that they tested.

[1] Accepted for publication 26 May 1999.

Schedorhinotermes lamanianus (Sjöstedt), an African rhinotermitid, secretes a feeding stimulant from labial glands that leads to aggregations at a feeding site (Reinhard & Kaib 1995). This water-soluble, heat-resistant (100°C for 14 h) secretion is thought to be persistent (Reinhard et al. 1997). Tests with labial gland secretions have demostrated that stimulation of termite feeding activity is lost if the substrate drys but attractivity returns upon rewetting (Rheinhard & Kaib 1995). A feeding stimulant also is secreted by the labial glands of *Reticulitermes santonensis* Feytaud (Reinhard et al. 1997). These examples suggest that previously fed-upon baits within a detection, monitoring, or bait station may be more appealing than untouched stations and have the potential to promote their reuse by the same or other termite populations.

In contrast, termite activities also could be disrupted at a feeding site. Directions for use of termite baits can include shaking termites from a monitoring station into a baiting matrix or, with other designs, a station is placed over a shelter tube or at a manually broken, active shelter tube (Potter 1997). Agitation could cause a release of alarm pheromones or other responses that could repel termites away from the site (Ernst 1959, Moore 1969, Maschwitz & Mhhlenberg 1972, Wilson & Clark 1977, Stuart 1981, Kaib 1990, Roisin et al. 1990). There is also the possibility that termites may be accidentally killed during transfer or tube breaking, which may serve as a deterrent to further activity. It is unknown how long these types of disruption may affect termite behavior, but they should be an important consideration in termite baiting programs. In spite of the potential implications to the use of a technology recently registered by the United States Environmental Protection Agency (Potter 1997), there have been no published studies on the reuse of detection, monitoring, or bait stations by subterranean termites.

We conducted a series of assays intended to examine reuse of subterranean termite detection, monitoring, or bait stations by the same or different termite populations by measuring corrugated cardboard consumption rates. Termites were simultaneously provided a choice of stations that were previously exposed to nestmates, conspecifics, allospecifics, and stations with no previous termite contact. Herein, we report results from these choice-feeding assays by using three populations of two termite species *Reticulitermes flavipes* (Kollar) and *R. virginicus* Banks to examine their preference to stations with or without previous termite contact.

Material and Methods

Termites. Three populations each of *R. flavipes* and *R. virginicus* were removed from infested logs (located at least 100 m apart) at the Westbrook Farm in Spalding County, Georgia, by using the techniques described by La Fage et al. (1983). Alates from each population were used to identify species (Weesner 1965). Termites were maintained in separate, clear plastic boxes (20 by 24 by 10 cm) with lids maintained in an unlit environmental chamber at 24°C for 2 to 4 mo prior to bioassay. Boxes were lined with damp wood (*Pinus* spp.) slats measuring . mm by 2 cm by 10 cm and Whatman #1 filter paper disks moistened with distilled water.

Feeding stations. The feeding stations used were commercially available termite stations called the Firstline™ GT Termite Bait Station (FMC Corp., Princeton, New Jersey). Stations consisted of 50-ml plastic-lidded centrifuge tubes each with 8, 3-mm holes drilled around the circumference in four columns. One hole was enlarged on each station to allow attachment of a piece of 3-mm (OD) Tygon® tubing. Each station contained one coiled piece of corrugated cardboard 15.2 by 9.4 by 0.2 cm.

We consistently prepared more stations and corrugated cardboard inserts than needed for bioassay to ensure adequate materials. Ninety corrugated cardboard pieces were oven dried for 1 h at 65°C and placed in a desiccator for 0.5 h before being weighed and individual weights recorded. Before bioassay, each corrugated cardboard piece was moistened with 3.5 ml of distilled water (90% wt:wt) and placed into separate feeding stations.

Feeding station preconditioning. Twelve groups of 200 worker termites from each population were placed into separate feeding stations that were covered with lids. The mean weight per termite for each group and total termite weight per group was calculated. Twenty-six feeding stations were prepared without termites to provide undamaged stations for the choice assay. Each station was placed in a clear plastic container (16 by 11 by 5 cm) with a lid. Boxes were lined with 60 g of sand moistened with 10 ml of water. Termites were provided egress, through the holes in each station, to the moistened sand in the box. Stations were maintained in an environmental chamber at 27°C and dismantled on the 18th d following initiation of the preconditioning period. Live termites were counted and mean and total weights of termites measured for each station. Corrugated cardboard pieces were dried for 1 h at 65°C, brushed clean, and placed in a dessicator for 0.5 h. Corrugated cardboard pieces then were reweighed and corrected according to weight change in unconditioned controls. Consumption rate (milligrams per gram of termite per day) was calculated based on termite survival, average weight per termite in each station, and corrected weight change of corrugated cardboard (Su & La Fage 1984). Corrugated cardboard pieces were returned to the same feeding station used in preconditioning and this was the unit examined as a treatment in subsequent bioassays.

Choice assay. Following preconditioning, potential preferences of the same three *R. flavipes* or three *R. virginicus* populations to feeding stations with previous termite contact were tested. Termites were concurrently offered stations that had previous contact with nestmates, conspecifics, or allospecifics, or that had no previous termite exposure.

Twenty clear plastic boxes (20 by 24 by 10 cm) were used as arenas for this bioassay. Boxes were lined to a depth of 5 mm with 240 g of sand moistened with 40 ml of distilled water. An introduction chamber was situated in the middle of each arena. The introduction chamber was a 4.5-cm round container, 3.5-cm in height, lined with filter paper moistened with distilled water. Four sections of Tygon tubing (2mm ID, 7.5 cm in length) positioned equidistant around the circumference of the container connected the introduction chamber to one of each of the four feeding stations (nestmate, conspecific, allospecific, no contact). Each connecting tube was clamped shut to allow termites to acclimate to the introduction chamber, before allowing them to disperse to the feeding-station treatments. Three replicates of 1,000 termites for each termite population were placed into separate introduction chambers within each arena. After 2 h, the tubing was unclamped to allow termites to move to the feeding stations. From the feeding stations, termites were allowed access to the entire arena through the holes provided in each station. Three arenas were prepared with no termites (controls) for measuring corrugated cardboard consumption rates.

Arenas were placed in an environmental chamber at 27°C for 18 d in complete darkness. At the end of the test period, arenas were dismantled and the number of live termites in each feeding station and those in the rest of the arena were recorded. Mean termite weight and total weight of termites per arena were measured. Corrugated cardboard from each feeding station was reweighed after drying for 1 h at 65°C, brushed clean, and placed in a desiccator for 0.5 h. Weight was corrected according to weight change of unconditioned controls. Cardboard consumption for each monitoring station per gram of termite per day was calculated based on termite survival, average weight per termite, and corrected weight change of cardboard for each monitoring station treatment (Su & La Fage 1984).

Analysis of variance (ANOVA) was performed to compare mean corrugated cardboard consumption rates by population and species for both the preconditioning and choice-test data. In addition, treatments were blocked on population and species for the combined data set ANOVA and means separated using the protected least significant difference (LSD) test (SAS Institute1990).

Results and Discussion

Corrugated cardboard consumption rates during the preconditioning period averaged 20.14 mg/g/d for *R. flavipes* and 21 mg/g/d for *R. virginicus*. Only preconditioned cardboard pieces having between 10% and 20% of the cardboard consumed were used in the choice test assays. Corrugated cardboard consumption rate from preconditioning had no significant effect on consumption rate during the choice assay as indicated by ANOVA of the combined data ($P = 0.14$). The entire data set is presented in Tables 1 and 2. Total corrugated cardboard consumption rates, during the choice-test bioassay, varied from 5 to 19 mg/g/d among *R. flavipes* and from 7 to 36 mg/g/d among *R. virginicus* (Tables 1 and 2). Preferences varied from replicate to replicate for all populations tested. Although a preference was indicated within certain replicates, combining data by population and species (Table 3) resulted in no significant differences in mean cardboard consumption rates when comparing the species or combined data sets. Although analysis of the data at the population level provided statistical differences between some treatments for *R. flavipes* populations 1 and 3, we suggest these differences are of little biological significance due to the variability at the population level (Table 1).

It was expected, based on the results of Delaplane & La Fage (1989a) and Reinhard & Kaib (1995, 1996), that termites would show a preference toward stations with previous termite contact. Although termites made contact with all feeding stations within 1 min of release from the introduction chamber, we were unable to count the initial number of termites that traveled to each station. Therefore, we could not determine if there was a preference for feeding stations that were visited first by the most termites. However, we assumed that because termites were free to travel between stations within each arena, over time they would examine each feeding station. Oi et al. (1996) indicated the importance of time and spatial separation of treatments for determination of preference in ter-

Table 1. Cardboard consumption rate for *R. flavipes* by population, replicate, and treatment from feeding station choice tests.

Treatment/ replicate[a]	Cardboard consumption rate[b]		
	Pop. 1	*Pop. 2*	*Pop. 3*
R1			
NM	3.114	0	0
CS	5.045	12.546*	2.126
AS	5.559	0	1.155
NO	5.148	0	2.237
R2			
NM	3.067	0	0
CS	0.380	1.437	0
AS	3.359	7.854*	1.831
NO	7.916*	3.129	3.890*
R3			
NM	5.929	2.448	0
CS	2.110	4.997*	2.644
AS	2.324	0.714	1.254
NO	6.464	0	1.877

*Indicates a preferred treatment within a replicate based on the criteria of one cardboard consumption rate being twofold higher than the other three rates within the same replicate.
[a]NM, nestmate; CS, conspecific; AS, allospecific; NO, no previous termite exposure.
[b]In milligrams of cardboard consumed per gram of termite per day.

mite choice assays. Our time frame of 18 d should have been sufficient for each group of 1,000 termites to indicate a preference between the feeding stations available to each group of termites.

Reinhard & Kaib (1995) demonstrated that a persistent labial gland secretion has a positive effect on termite feeding and aggregations. In addition, Reinhard & Kaib (1996) demonstrated that *R. santonensis* was able to detect labial gland secretions of eight species representing five families of termites. Thus, we anticipated that chemical cues left on the cardboard from the preconditioning period would be a factor in our feeding-preference tests. Our data appear contrary to that assumption in that no preference was shown for any particular treatment as indicated by the combined data (Table 3).

The entire data set was presented in Tables 1 and 2 to illustrate that although data from termite-feeding choice tests may show no preference (Table 3), a single group of termites (replicate) can, and does, apparently preferentially feed at one or two sites. Because particular preferences were often not repeated by other groups of termites within a test, the combined data could indicate that no preference was displayed (Tables 1–3). If termite choice test data show no preference, one may conclude that termites distribute equally between those choices presented during bioassay. We suggest that when combined data indicate no preference it simplifies the complex nature of the feeding-site selection process used by subterranean termites.

Table 2. Cardboard consumption rate for *R. virginicus* by population, replicate, and treatment from feeding station choice tests.

Treatment/ replicate[a]	Cardboard consumption rate[b]		
	Pop. 1	*Pop. 2*	*Pop. 3*
R1			
NM	5.960	6.515	4.509
CS	9.280	23.509*	0
AS	15.882	6.838	0.111
NO	4.866	6.833	4.397
R2			
NM	5.606	3.630	0
CS	10.380	6.417	3.755
AS	10.308	11.918	1.986
NO	9.078	3.814	1.367
R3			
NM	4.858	7.778	0.666
CS	2.944	1.249	0
AS	4.328	22.087*	2.021
NO	4.063	2.034	4.584*

*Indicates a preferred treatment within a replicate based on the criteria of one cardboard consumption rate being twofold higher than the other three rates within the same replicate.
[a]NM, nestmate; CS, conspecific; AS, allospecific; NO, no previous termite exposure.
[b]In milligrams of cardboard consumed per gram of termite per day.

Examination of the data by replicate may provide insights into that process. There are two possible trends in these data indicative of a termite response to chemical cues left on food resources that deserve additional experimentation. The first speculation points to a resource-use strategy that *Reticulitermes* may practice during competition for food resources. If our treatments are ranked within a replicate and compared across all replicates, then the least-favored treatment was the nestmate treatment and the most-often favored treatment was the allospecific treatment. When examined in this way these data indicate that *Reticulitermes* are more likely to colonize food resources identified as having been used (visited) by another species before those containing a nestmate signal. The second speculative trend points to a different resource use strategy between the two species tested. If we assume that a treatment is determined to be preferred when one treatment, within a replicate, provided at least twice the feeding rate of the other three treatments within that replicate, we see a potential difference between the two species. That trend would indicate *R. flavipes* is most likely to prefer one feeding site to the exclusion of others (in five out of nine replicates) compared with *R. virginicus* (three out of nine replicates) (Tables 1 and 2).

The lack of response to potential chemical cues seen in our assays could have been the result of drying the cardboard, especially if any of the cues are volatile. It was our contention that drying the cardboard to obtain more consistent cardboard consumption rates was more important then loosing potential chemical

Table 3. Mean (± SE) cardboard consumption rates by treatment for the population, species, and combined data from the feeding station preference bioassays.

Treatment/ replicate[a]	Cardboard consumption rate[b]		
	R. flavipes Pop. 1	*R. flavipes* Pop. 2	*R. flavipes* Pop. 3
NM	4.037 ± 0.946a	0.072 ± 0.816a	0 ± 0b
CS	2.510 ± 1.362b	6.327 ± 3.275a	0.881 ± 0.809ab
AS	3.747 ± 0.954ab	2.856 ± 2.507a	1.413 ± 0.211ab
NO	6.509 ± 0.799a	0.624 ± 1.043a	2.668 ± 0.620a
	R. virginicus Pop. 1	*R. virginicus* Pop. 2	*R. virginicus* Pop. 3
NM	5.475 ± 0.325a	5.974 ± 1.228a	1.288 ± 1.405a
CS	7.535 ± 2.317a	10.392 ± 6.726a	0 ± 0a
AS	10.173 ± 3.336a	13.614 ± 4.483a	1.373 ± 0.631a
NO	6.002 ± 1.555a	4.229 ± 1.402a	3.449 ± 1.043a
	R. flavipes total	*R. virginicus* total	Combined total
NM	0.917 ± 0.932a	4.246 ± 0.964a	1.288 ± 1.320a
CS	3.240 ± 1.375a	5.924 ± 2.666a	4.932 ± 2.885a
AS	2.672 ± 0.848a	8.387 ± 2.441a	5.535 ± 3.014a
NO	2.267 ± 0.980a	4.560 ± 0.774a	3.984 ± 1.265a

Means followed by the same letter in each column under each heading are not significantly different ($P < 0.05$, LSD test).

[a]NM, nestmate; CS, conspecific; AS, allospecific; NO, no previous termite exposure.

[b]In milligrams of cardboard consumed per gram of termite per day.

cues, especially because of the heat-resistant qualities of the labial gland secretions (Reinhard & Kaib 1995). However, our choice of cardboard, with its corrugations, provided numerous thigmotactic cues that could have confounded or masked the importance of any chemical cues. The importance of thigmotactic cues provided by cardboard cannot be overlooked when examining the results of our bioassays. Delaplane & La Fage (1989a) demonstrated that there was a weak or absent response to hexane extracts of termite-damaged wood and concluded that there was little termite response to chemical cues. Therefore, Delaplane & La Fage (1989a) stated that only thigmotaxic cues were involved in their feeding-preference tests.

What are the factors termites use to determine a suitable feeding site and how are others recruited to that site? Termite determination of a suitable feeding site may involve the interaction of numerous cues, including chemical and thigmotactic. Additional inputs could include moisture content (Delaplane and La Fage 1989b), temperature of the food source (Haverty & Nutting 1974), as well as termite population vigor and density (Lenz & Barrett 1984, Lenz 1985). Our choice-test bioassays indicate previous exposure to one group of *Reticulitermes* does not preclude colonization of that site by another, different group of *Reticulitermes*. Therefore, we suggest that termite detection, or monitoring stations can be reused in a termite bait control strategy or in long-term studies of termite biology involving *R. flavipes* and *R. virginicus*.

Acknowledgment

We would like to thank Monica Townsend and Sherry Ridgeway for collecting termites and assisting in these experiments.

References Cited

Delaplane, K. S. & J. P. La Fage. 1989a. Preference of the Formosan subterranean termite (Isoptera: Rhinotermitidae) for wood damaged by conspecifics. J. Econ. Entomol. 82: 1363–1366.

1989b. Preference for moist wood by the Formosan subterranean termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 82: 95–100.

Ernst, E. 1959. Beobachtungen biem spritzakt der *Nasutitermes*-soldaten. Rev. Suisse Zool. 66: 289–295.

Forschler, B. T. 1996. Baiting *Reticulitermes* (Isoptera: Rhinotermitidae) field colonies with abamectin and zinc borate-treated cellulose in Georgia. Sociobiology 28: 459–484.

Haverty, M. I. & W. J. Nutting. 1974. Natural wood-consumption rates and survival of a dry-wood and a subterranean termite at constant temperatures. Ann. Entomol. Soc. Am. 67: 153–157.

Kaib, M. 1990. Intra- and interspecific chemical signals in the termite *Schedorhinotermes* - Production sites, chemistry, and behaviour, pp. 26–31. *In* F. G. Gribahin, K. Wiese, & A.V. Popov [Eds.], Sensory systems and communication in arthropods. Advances in Life Sciences. Birkhauser, Basel.

La Fage, J. P., N.-Y. Su, M. J. Jones & G. R. Esenther. 1983. A rapid method for collecting large numbers of subterranean termites from wood. Sociobiology 7: 305–310.

Lenz, M. 1985. Variability of vigour between colonies of *Coptotermes acinaciformis* (Froggatt) (Isoptera: Rhinotermitidae) and its implication for laboratory experimentation. Bull. Entomol. Res. 75: 13–21.

Lenz, M. & R. A. Barrett. 1984. Implications for comparability of laboratory experiments revealed in studies on the effects of population density on vigour in *Coptotermes lacteus* (Froggatt) and *Nasutitermes exitiosus* (Hill) (Isoptera: Rhinotermitidae, Termitidae) and its implication for laboratory experimentation. Bull. Entomol. Res. 74: 477–485.

Maschwitz, U. & M. Mühlenberg. 1972. Chemische gefahrenalarmierung bie termite. Naturwissenschaften 59: 516–517.

Moore, B. P. 1969. Biochemical studies in termites, pp. 407–432. *In* K. Krishna & F. M. Weesner [Eds.], Biology of termites, vol. 1. Academic Press, New York.

Oi, F. M., N.-Y. Su, P. G. Koehler & F. Slansky. 1996. Laboratory evaluation of food placement and food types on the feeding preference of *Reticulitermes virginicus* (Isoptera: Rhinotermitidae). J. Econ. Entomol. 89: 915–921.

Potter, M. F. 1997. Termite baits: a status report. Pest Control Technol. 22 (2): 24–26, 28, 30, 35-37, 97, 105, 106.

Reinhard, J. & M. Kaib. 1995. Interaction of pheromones during food exploitation by the termite *Schedorhinotermes lamanianus*. Physiol. Entomol. 20: 266–272.

1996. A feeding stimulating signal in the labial gland secretion of the termite *Reticulitermes santonensis* Sjöstedt (Isoptera: Rhinotermitdae). *In*: Proceedings of the 20th International Congress of Entomology.

Reinhard, J., H. Hertel & M. Kaib. 1997. Feeding stimulating signal in the labial gland secretion of the subterranean termite *Reticulitermes santonensis*. J. Chem. Ecol. 23: 2371–2381.

Roisin, Y., C. Everaerts, J. M. Pasteels & O. Bonnard. 1990. Caste-dependent reaction to soldier defensive secretion and chiral alarm/recruitment pheromone in *Nasutitermes princeps*. J. Chem. Ecol. 16: 2865–2875.

SAS Institute. 1990. SAS/STAT user's guide, ver. 6, 4th ed. SAS Institute, Cary, North Carolina.

Stuart, A. M. 1981. The role of pheromones in the initiation of foraging, recruitment and defense by the soldiers of a tropical termite, *Nasutitermes corniger* (Motschulsky). Chem. Senses 6: 409–420.

Su, N.-Y., & J. P. La Fage. 1984. Comparison of laboratory methods for estimating wood consumption rates by *Coptotermes formosanus* (Isoptera: Rhinotermitidae). Ann. Entomol. Soc. Am. 77: 125–129.

Su, N.-Y. & R. H. Scheffrahn. 1988. Intra- and interspecific competition of the Formosan and the Eastern subterranean termite: evidence from field observations (Isoptera: Rhinotermitidae). Sociobiology 14: 157–164

1996. A review of the evaluation criteria for bait-toxicant efficacy against field colonies of subterranean termites (Isoptera). Sociobiology 28: 521–530.

Weesner, F. M. 1965. The termites of the United States: A handbook. The National Pest Control Association, Elizabeth, New Jersey.

Wilson, D. S. & A. B. Clark. 1977. Above ground predator defense in the harvester termite *Hodotermes mossambicus* (Hagen). J. Entomol. Soc. South Africa 40: 271–282.