

## Effects of Disturbance-Induced Trauma on Foraging by Subterranean Termites (Isoptera: Rhinotermitidae)<sup>†</sup>

by

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### ABSTRACT

Toxicant baiting systems are effective at population suppression against both the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, and the Eastern subterranean termite, *Reticulitermes flavipes* (Kollar). However, trap shyness (*i.e.*, station abandonment) is often quoted as a confounding factor affecting their success. We observed that *C. formosanus* field colonies occasionally abandoned established research field monitors when disturbed. We hypothesized that inadvertent trauma caused by trap disturbance could be a contributing factor to this abandonment phenomenon. We investigated the effects of the presence of physically-traumatized workers and soldiers on the consumption of food sources by *C. formosanus* and *R. flavipes* in a laboratory choice assay. Feeding was significantly reduced on food sources in contact with dead termites in laboratory trials with both termite species. Our results suggest that there is a continuum of behavioral interactions with dead nest mates, starting with anti-feedant effects and eventually, abandonment and walling-off of the dead termites and the source of mortality. Baiting protocols need to minimize disturbances that could cause trauma and subsequent avoidance of field monitors and baits.

**Key Words:** Disturbance, Foraging behavior, Nestmate interactions, Termite

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<sup>†</sup>This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or recommendation for use by the USDA Forest Service.

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## INTRODUCTION

The most confounding aspect of subterranean termite management is that colonies are cryptically located, making direct control all but impossible (Kofoid 1934). To this end, many studies have been conducted to elucidate their behavior in both natural and urban habitats (Stuart 1988; MacDonald *et al.* 1999; Eggleton *et al.* 2002). Specifically, knowledge of foraging behavior has allowed us to utilize the foraging worker population as a means of introducing a toxicant to the colony at large (French 1994; Grace *et al.* 1996; Pawson & Gold 1996; Su & Scheffrahn 1998). The goal of baiting technology is to reduce, and ideally eliminate termite populations and minimize the possibility of structural infestation.

A common method of subterranean termite baiting involves two distinct processes: monitoring (detection), and toxicant baiting. Monitoring involves installing in-ground stations around structures, into which some form of cellulose substrate is placed. Periodic checks are made to determine whether termites are actively feeding on the monitors. Once termites have begun using the monitor resource, it is switched with another cellulosic material containing a toxicant. Termites then begin consuming the toxicant-containing substrate, which in turn is partially redistributed to other termites in the colony via trophallaxis.

This baiting procedure involves an inevitable degree of disturbance of foraging termites. The process of monitoring termite activity involves baits/monitors being removed and examined for activity, damage, and need of replacement. It is generally accepted that termites will rapidly abandon nest or food sources in response to disturbance (Noirot & Darlington 2000). However, this egress is usually temporary, with ingress into the area of disturbance occurring within seconds or, at most, hours (Schwinghammer & Houseman 2006; Hu *et al.* 2003). We have observed that Formosan subterranean termite (*Coptotermes formosanus* Shiraki) foragers will sometimes totally abandon traps (Tamashiro *et al.* 1973) and field monitors that have previously been active for many years. This phenomenon was associated with the release of termites (living and dead) that had been in captivity for at least 1-wk, along with their carton material and other debris.

We hypothesized that termites returned to stations were stressed by conditions during captivity, producing mortality. We sought to document

whether this phenomenon could be replicated under controlled laboratory conditions. As dead individuals were present among the stressed captive termites being returned to our field colonies, we hypothesized that the presence of dead/moribund or stressed termites were having a detrimental effect on foraging behavior in both bait stations and traps. We conducted laboratory experiments with *C. formosanus* and *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae) to evaluate the anti-feedant and behavioral effects of dead/moribund termites placed in jars with food materials, while keeping other disturbance factors equal.

## METHODS

### Test arena

Arenas consisted of three 473 ml polystyrene jars (Qorpak, Bridgeville, PA) connected with 4 cm lengths of flexible tubing (12.7 mm O.D., 9.5 mm I.D., Tygon, Performance Plastics, Akron, OH) sealed with silicone sealant (100% Silicon Rubber Sealant, Dap Inc, Baltimore, MD). The tubing was placed such that 1 cm extended into each jar, leaving a 2 cm distance between each pair. Plastic drinking straws (Solo Flexible Straws, Solo Cup Company, Urbana IL) were inserted into the openings of the tubes and leaned against the sides of the jars. Into each jar was placed 150 g silica sand (40 - 100 mesh, Thermo Fisher Scientific, Waltham MA) and 30 ml distilled water (27 ml for *R. flavipes*; Fig. 1). Drinking straws were then removed leaving passages from the connecting tubes to the sand surface to facilitate rapid discovery of the food sources in the outer jars.

Wooden wafers were placed in each of the outer jars, and termites were placed in the center jar of each arena (details below). Arenas were kept in unlighted incubators ( $28 \pm 1^\circ\text{C}$ ;  $25 \pm 1^\circ\text{C}$  for *R. flavipes*;  $\sim 70\%$  R.H.) for 5 days to allow for universal discovery of both outer jars. After this equilibration period, the initial wooden blocks in the outer jars were removed and thrown away and treatments were applied to randomly-selected outer jars. Prior to initiation, treatment wooden blocks were placed into Petri dishes, autoclaved, dried for 24 hrs at  $90^\circ\text{C}$ , and cooled for one hour under desiccant conditions and weighed.

Dead/moribund termites were generated by crushing live workers (from the same colony as the original initiation) between two small Petri dishes.

The original unweighed blocks were tapped to remove termites, and then discarded. These termite remains were scraped from the Petri dishes and placed into a randomly selected (by coin toss) outer jar for each replicate near the opening of the tunnel (near but not precisely where the wood block was sitting). After five minutes, pre-weighed wooden wafers were placed in the center of the outer jars of all arenas; within treatment jars, wafers were placed on top of the dead termites.

Semiweekly observations were made of activity on the sand surface in each jar. Since termites below the surface of the sand, or within the confines of wood blocks would not be viewable, only those on the surface could be estimated. Observations were made by estimating the number of termites in each jar on a five point numerical scale ("0" = 0 termites, and 1-5 were assigned to activity levels of:  $\leq 20\%$ ,  $\leq 40\%$ ,  $\leq 60\%$ ,  $\leq 80\%$ ,  $\leq 100\%$ , respectively).

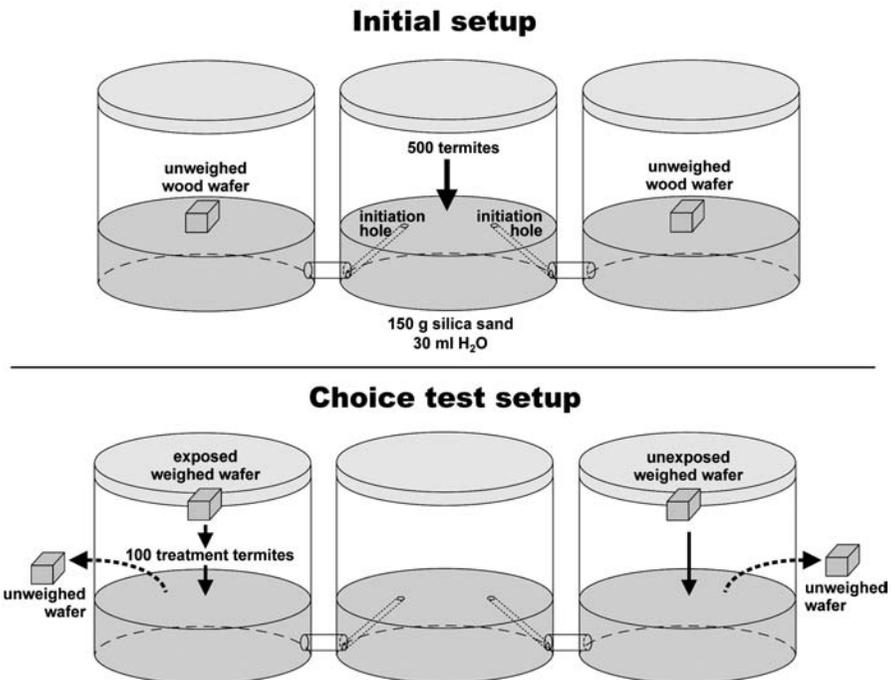


Fig. 1. Setup for necrophobia experiments (numbers refer to *C. formosanus* trials, see text for *R. flavipes* details).

### *Coptotermes formosanus*

Five hundred FST (10% soldiers: 90% workers) were placed in the center jar of each test arena, along with a 1 g ( $\sim 25.4 \times 12 \times 6.3$ mm) wafer of Douglas-fir [*Pseudotsuga menziesii* (Mirbel) Franco] sapwood in each of the two outer jars. All termites were collected from the same colony on the University of Hawaii at Manoa campus in pre-established traps (Tamashiro *et al.* 1973) immediately prior to the trial initiation. One hundred dead/moribund (treatment) workers (from the same colony) were placed into the pre-selected outside jars (Fig. 1). Two trials were conducted; the first contained 5 replicates and the second 7.

### *Reticulitermes flavipes*

Termites were collected from infested timber in a pine forest within 10 miles of Starkville MS (Noxubee Wildlife Refuge maintained by the US Fish and Wildlife Service). Infested lumber was sectioned into 0.5-1 m lengths and placed into  $\sim 114$  liter (30 gal.) metal trash cans and returned to the laboratory. Trash cans were kept at ambient lab temperatures ( $\sim 24^\circ\text{C}$ ). Identification of termites used soldier morphology following the key of Hostettler *et al.* (1995) as *R. flavipes*. Termites were collected from the field 6 weeks prior to the beginning of the study.

Arenas were prepared as described above, with ten arenas prepared for the study. Silicone caulk (GE Silicone II 100% silicone window and door sealant, General Electric, Huntersville NC) was used to seal the tubes between each arena jar. Pieces of aluminum flashing tape (Nashua tape, Tyco Adhesives, Franklin KY) were placed over each hole and cut to insert the tubes, with silicone caulk sealing the tubing to the tape. Jars were secured to a piece of Plexiglas ( $20.32 \times 40.64$  cm) using tape.

Southern yellow pine blocks (*Pinus* Linn. *spp.*; nominal  $1.3 \times 1.3 \times 1.3$  cm) were placed immediately next to the opening of the sand tunnels in the outer jars. Ten groups of 200 termites (198 workers and 2 soldiers) were placed into the center jar of individual arenas. Each treatment consisted of 40 nestmates (workers only; freshly collected from the same original colony) crushed as described above. On day 19 arenas were quickly split into three trays, each containing one jar (tubing going with the outer jars), and the surviving workers and soldiers in each jar were counted and recorded. Wood

blocks were removed, scraped clean of sand and termite excrement, and dried and weighed.

### Analysis

It was hypothesized that consumption of control wafers would exceed that of wafers in contact with dead/moribund termites. The experimental design was such that each individual arena contained a control jar (no dead termites added) and a treatment jar. Comparisons could then be paired in analysis. One-tailed paired t-tests were conducted to resolve specific treatment differences in wood consumption (SAS Institute 1997).

## RESULTS

### *Coptotermes formosanus*

Direct observation of termite behavior in jars was equivocal (Fig. 2). Termite workers initially avoided the injured termites, while soldiers seemed to congregate, however there was no measurable difference in activity levels in the jars.

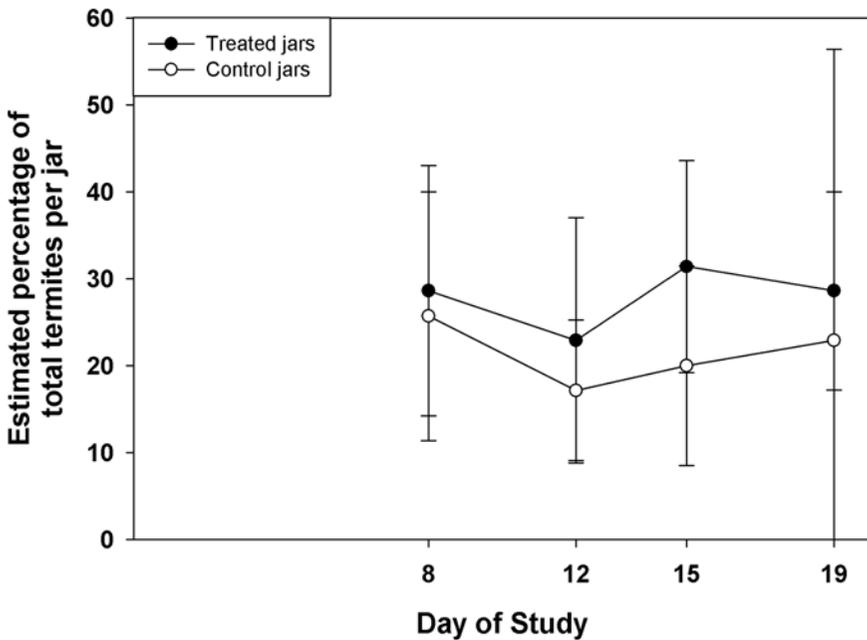


Fig. 2. Estimated percentage of total *C. formosanus* termites observed on the sand surface in each of the outer jars (one each control and treatment) of a choice assay.

Wood consumption was negatively affected by the presence of the dead termites in two separate laboratory choice assays with *C. formosanus*. The first assay produced a mean consumption of  $0.213 \pm 0.032$  g and  $0.372 \pm 0.040$  g for the exposed and unexposed jars, respectively (Paired one-tailed t-test;  $T_4 = -2.37$ ;  $P = 0.038$ ); the second trial produced consumption means of  $0.244 \pm 0.050$  g and  $0.485 \pm 0.048$  g for the exposed and unexposed jars, respectively (Paired one-tailed t-test;  $T_6 = -2.48$ ;  $P = 0.0239$ ).

### *Reticulitermes flavipes*

Observations of termite presence within the various jars (Fig. 3) indicated that the treatment did not completely repel termites at any point during the study. Mean mortality in the arenas was  $12.85 \pm 1.04\%$  (range: 9 to 18.5%). At the study's end, little or no repellency was noted in the treated jars as the mean percentage of survivors found in the treated jars was  $23.97 \pm 8.08\%$  (range: 0 to 85.96%). Mean wooden block loss was  $0.117 \pm 0.010$  g for jars where dead termites were added and  $0.172 \pm 0.009$  g for the control jars.

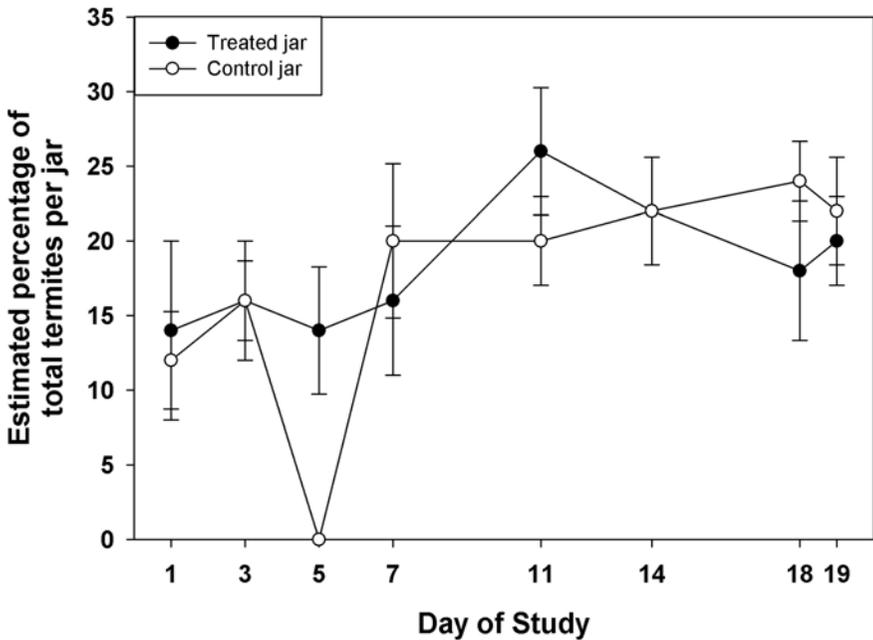


Fig. 3. Estimated percentage of total *R. flavipes* termites observed on the sand surface in each of the outer jars (one each control and treatment) of a choice assay.

Significantly more wood was lost from blocks in the control jars compared to the treated jars (Paired one-tailed t-test;  $T_9 = -2.99$ ;  $P = 0.008$ ).

## DISCUSSION

Our results with both termite species consistently showed that there was a significant negative effect of the presence of dead or moribund workers on feeding. Su *et al.* (1982) found that an accumulation of large numbers of dead and decaying termites in soil treated with delayed toxicants caused foraging termites to wall-off and avoid the area. Initially, Su *et al.* (1982) suggested that “factors” emanating from the dead termites may have caused the avoidance behavior. Although necrophobia could be related to the feeding effects in this study, the effect is more subtle than the avoidance behaviors discussed by these authors.

Su *et al.* (1995) offered an additional explanation for this phenomenon, e.g., as learned feeding deterrence associated with ingestion of sublethal doses of a delayed-toxicant (sulfuramid in this instance). Similar observations were reported by Su & Scheffrahn (1988). Associative learning, *i.e.*, “the acquisition of conditioned responses by the Pavlovian association of rewards with previously meaningless stimuli” is common in social insects (Wilson 1971). In this case, however the association is not with a reward, but a negative stimulus; morbidity from toxicity of a chemical compound (the previously meaningless stimuli). In our study, however, the workers in the arenas did not receive any direct negative stimulus that could cause learned avoidance. Our results suggest that a direct stimulus (*i.e.*, sublethal dosage of a toxicant) is not necessarily needed, and that similar behavior can be elicited by mere contact with dead/moribund nest mates (an adaptation to avoid indirect contact with the source of mortality).

Rosengaus *et al.* (1999) discussed how *Zootermopsis* sp. infected with fungal pathogens are able to signal alarm via a combination of a vibrational and pheromonal cues to nestmates that in turn flee to avoid the contaminated area. Campora & Grace (2007) also observed a related behavioral phenomenon. These authors found that termites in a two-dimensional arena would avoid areas of the arenas in which borate-treated wood had been placed. What was even more compelling was that upon switching the wood to the opposite side of the arena, feeding continued briefly on the now-treated woods and

avoidance was delayed, indicating that the termites were not being repelled by the borate treatment directly but by some secondary phenomenon. In this case, sublethal effects from the toxicant may have accounted for the learned avoidance rather than necrophobia, since termites did not die in the vicinity of the treated wood due to the slow action of the borate toxicant.

Although the underlying mechanisms that are responsible for these long-term avoidance behaviors are still largely unknown, there is a great deal of published work on short-term alarm factors. Short-term alarm responses are often mediated by compounds that could cause feeding deterrence. Stuart (1970) discussed the chemical alarm response of *Zootermopsis nevadensis* (Hagen). He found that while there was no volatile compound detected, there was evidence for a material that may require direct contact for detection, and/or direct physical vibrations of the substratum. Reinhart & Clément (2002) reported that alarm reactions could be generated by crushed soldier heads, as observed in various *Reticulitermes* spp. Various compounds associated with the soldier frontal gland are present in the head and it was assumed that these compounds could be involved in the responses to the whole soldier heads.

Schwinghammer & Houseman (2006) investigated the alarm response of *R. flavipes* to a burst of air. They reported that alarm responses usually consisted of an immediate partial evacuation followed by a movement back into the site that eventually resulted in more termites present after the alarm than before. In their study, as soldier proportions increased fewer termites evacuated areas following disturbances, suggesting that the alarm “factor” (pheromone or vibrational cue) was reduced by the presence of soldiers (Schwinghammer & Houseman 2006). Hu *et al.* (2003) found that various vibrational frequencies caused *R. flavipes* and *C. formosanus* workers and soldiers to withdraw from the source of vibration, but that this response was limited to periods ranging from ca. 80 to 200s. As these alarm responses are relatively short-lived, other factors are likely involved with the longer-term occurrence observed in our results. It is possible that the crushed workers released a pheromone that caused the anti-feedant effects.

The pest control industry is understandably interested in reducing the labor time associated with termite baiting, while our results indicate that there is also a biological basis for minimizing inspection events. If termite mortality occurs in a monitoring station, feeding rates can decrease, although

there may be no obvious decrease in the numbers of termites in the station. Additionally, a decrease in feeding could have a dramatic effect on toxicant consumption rates and thus time-to-success in baiting studies. Conversely, our results suggest that bait consumption when used as a measure of bait success can be confounded by station disturbance. Although bait consumption has been used, activity level seems to be the most readily reported measure of overall success in recent termite baiting field studies (Su & Scheffrahn 2000). We contend that activity in a monitoring station is a more definitive measure of baiting success than bait consumption, especially when there is no consideration for potential disturbance effects.

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