

Value of Observational Data in Bioassays for Evaluating Bait Efficacy Against Subterranean Termites (Isoptera: Rhinotermitidae)

by

Thomas G. Shelton¹ & J. Kenneth Grace¹

ABSTRACT

A novel method is presented for determining bait removal and acceptance of up to nine choices under laboratory conditions by the Formosan subterranean termite, *Coptotermes formosanus* Shiraki. Data from a study using this method showed that estimated bait removal and bait mass loss data were correlated ($r = 0.694$; $P < 0.0001$), indicating the value of periodic bait removal estimates during choice studies. We also provide data from a two-choice test which allows collection of standard data such as mortality and wood mass loss at the end of the experiment, but also gives information on the daily progression of bait matrix removal by termites. We examined this method with *C. formosanus* using powdered cellulose bait matrices containing two rates each of zinc borate hydrate (ZB) or disodium octaborate tetrahydrate (DOT) paired with Ponderosa pine blocks. In the example trial, 2% DOT cellulose baits were removed by termites significantly more than all other baits during the first week of the experiment ($P = 0.001$), and overall mortality was significantly greater with DOT baits than with ZB or blank controls ($P < 0.0001$). Wood mass loss was significantly lower for those blocks paired with the DOT baits than for those paired with controls or ZB baits ($P < 0.0001$). No significant difference was found between either wood mass loss or mortality of termites exposed to 1% or 2% DOT bait. Thus by using the daily bait removal estimates, differences in rate performance between the two DOT baits could be assessed that were not apparent from standard quantitative data collected at the end of the experiment.

INTRODUCTION

Estimates of subterranean termite damage in the United States range from US \$100 million to over \$1 billion annually in damage, repair, and prevention costs (Su & Scheffrahn 1990). These estimates are conservative, as they are 10 years old, and based on data extrapolated from the 1980's (Mauldin 1982; Su & Scheffrahn 1990). New

¹Department of Plant and Environmental Protection Sciences, University of Hawaii, 3050 Maile Way, Honolulu, HI 96822-2271 USA

control methods for these species, such as baiting (Grace & Su 2000; Yates & Grace 2000), are based upon a low input of toxicants into the environment. Proper investigation of the efficacy of baiting materials has become an academic interest in providing support for the pest management industry.

In this paper we describe study methods used in our laboratory to investigate the foraging acceptability of a variety of bait matrices, as well as their efficacy when used with toxicants. In these studies we have employed mostly two-choice tests, although these methods have also been used to examine three-choice tests and forced feeding situations. We also present methods used in determining acceptance of bait matrices by subterranean termites in a large multiple choice arena. This arena is similar to the multiple choice chemometer of Rust & Reiersen (1977), except that we examined termite removal of foraging materials where Rust & Reiersen (1977) examined *Blattella germanica* (L.) movement among treated surfaces. We offer evidence that observational data can give results similar to mass loss measurements of bait consumption when collected carefully, similar to the estimation of bait consumption in the field by Forschler (1996).

Due to its economic importance (Su & Scheffrahn 1990; Pearce 1997; Grace & Su 2000; Yates & Grace 2000), our research has focused on the Formosan subterranean termite, *Coptotermes formosanus* Shiraki. The methods described here may also be applied to other species of subterranean termites, although adjustments are necessary to maintain temperature and humidity preferences of other species (Collins *et al.* 1973; Collins 1991; Rudolph *et al.* 1990; Woodrow & Grace 1998). Soldier caste proportions can be determined empirically or from literature values (Haverty 1977), to maintain natural caste proportions in these laboratory studies.

We developed these methods in response to the difficulties in assessing termite acceptance of powdery bait matrices in small-scale laboratory tests. Due to the nature of the matrices, mass loss was an inappropriate measure as baits were often built upon (both during rejection and feeding on the matrix), and occasionally the matrices were moved around the feeding arena without being consumed (TGS & JKG unpublished observations). Thus, we needed a method of assaying bait removal and acceptance that would better reflect termite behavior. We also needed a method applicable to both short-term and long-term feeding trials. As described here, we arrived at a method of assaying termite feeding on a daily basis, by estimating removal of the material daily. This method has been employed in bait *vs.* bait comparisons, as well as in bait *vs.* wood preference comparisons.

MATERIALS AND METHODS

Multiple choice arena materials

One type of assay we have employed several times in studying termite acceptance of bait matrices has been the syringe arena bioassay. These foraging arenas were set up to allow estimation of termite acceptance of three blank bait matrices. The arena consists of a 150 x 15mm plastic petri dish (360 ml volume; Fisher Scientific, Fair Lawn NJ), with nine holes punched to the diameter of a 1 ml (1 cc) tuberculin syringe (Becton-Dickinson corp., Rutherford NJ) at 40° arcs (Fig. 1). Nine tuberculin syringes with the needle fitting removed (*i.e.* only the syringe barrel and plunger remained) and the plunger pulled back to the 0.86 ml mark, were filled with one of the 3 bait matrices. Thus the total bait available from each syringe was 0.86 cm³. Masses of the baits were determined after oven-drying (90°C, followed with 1 hr of 0-2% RH while cooling), pre- and post-experiment. A single piece of 12.5 cm diameter Whatman #1 filter paper (Whatman, Haverhill, MA) was placed in the bottom of the plastic petri dish. Syringes containing the baits were placed in the nine holes as indicated in Fig. 1, and hot-glued in place.

While any subterranean termite might be used with this type of bait consumption test, we used *C. formosanus* to examine the effectiveness of these bait materials. Following a cooling period for the glue, the filter paper was moistened with 2 ml of distilled H₂O, and 400 *C. formosanus* (360 workers and 40 soldiers) were added in the center of the filter paper. Five replicates of the arena were made and placed into an unlit incubator at 28 ± 1°C, ~90% RH.

Termites were allowed to interact with the bait matrices for 24 hr, after which observations of bait removal were recorded (see Table 1), and the arenas dismantled. Baits were then removed from the syringes, dried as above, and their final masses determined. We examined the relationship between observational estimates of bait removal at 24 hr and dry mass losses. Even though the baits were packed equally, all three had different final packing masses, indicating differences in density between them (see results). Since there were differences among final packed masses, we needed to make relative comparisons of bait removal and chose percentage removal.

Multiple choice statistical analyses

This method was intended to give estimates of bait acceptance of various bait matrices. Since the data were not normally distributed, they were subjected to a Kruskal-Wallis test (Minitab 1999), and bait removal medians whose inter-quartile ranges (IQR's) did not overlap were considered significantly different if the Kruskal-Wallis test was

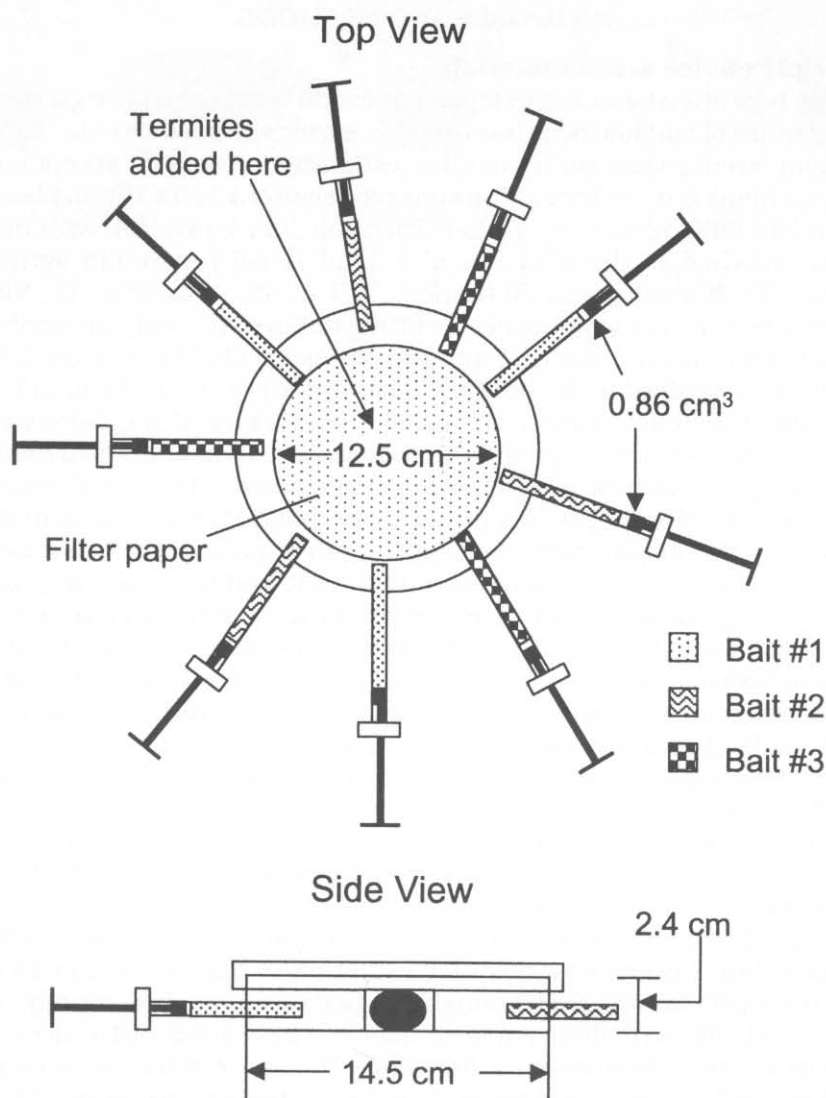


Fig. 1. Top and side views of the multiple choice experimental apparatus used for bait acceptance by subterranean termites.

significant. We examined the performance of our bait mass loss estimates, by comparing the observational data to the mass loss data. The association between the bait removal estimates and the percentage bait removal was investigated using Spearman's rank-order correlation (SAS Institute 1999).

Two-choice bait test materials

As an example of the two-choice method, we describe a short study to determine the effectiveness of two toxicants: disodium octaborate tetrahydrate (DOT), and zinc borate hydrate (ZB) in proprietary experimental bait matrices based upon powdered cellulose (Whitmire MicroGen, St. Louis MO). The comparisons tested were the toxicants (1.0 g containing either 1.0 or 2.0% DOT or ZB by weight in blank matrix) and blank bait matrix (as a control), each paired with a small (2 cm x 2 cm x 1.8 cm) lightly and evenly sanded block of *Ponderosa* pine [*Pinus*

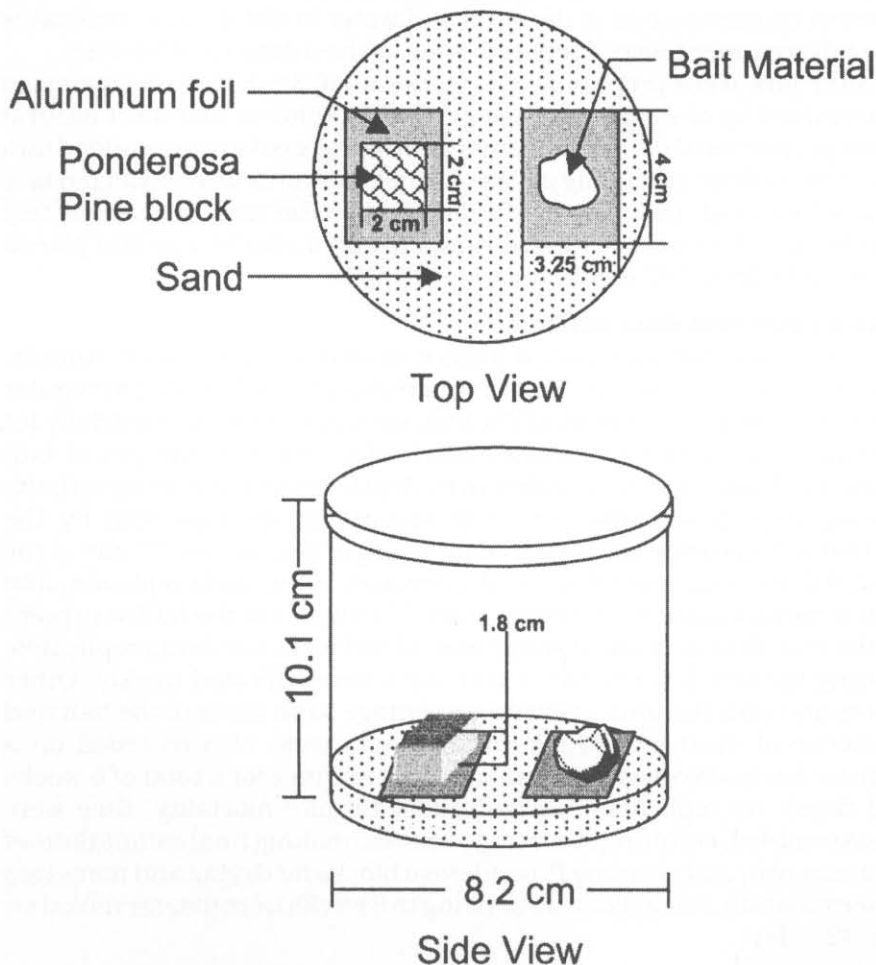


Fig. 2. Top and side views of the two-choice experimental apparatus for assaying bait acceptability with subterranean termites.

ponderosa Dougl. ex Laws.] as an alternative food source. Since wood consumption in this test was interpreted from mass loss, all blocks of *P. ponderosa* were numbered (in pencil), and their oven-dry (90°C for 24 hr, followed by 1 hr at 0-2% RH for cooling) masses were determined and recorded prior to use in the test. This study was performed in plastic screw top jars (10.1 cm x 8.2 cm diameter; Fig. 2) containing 150 g of washed silica sand (40-100 mesh; Fisher Scientific, Fair Lawn NJ) moistened with 30 ml of distilled H₂O. Bait materials and wood blocks were placed upon rectangles of aluminum foil (3.25 cm x 4.0 cm; Reynold's 667 Aluminum foil, Reynold's Metal Co., Richmond VA) to prevent contamination of the sand and water in the jar. Five replicates of each treatment were prepared prior to the addition of termites.

After jars were prepared, 360 workers (of 3rd instar or greater as determined by size and morphology) and 40 soldiers (to reflect natural caste proportions) of *C. formosanus* from a single colony were added into the center of the jars using a glass funnel. Termites were collected (see Tamashiro *et al.* 1973) from a field colony on the same day as the test was begun. Jars were arranged randomly on a plastic tray and placed in an unlit 28 ± 1°C incubator at ~90% RH.

Two-choice test data collection

Termite mortality and wood mass loss were recorded upon completion of the test at 6 weeks. Bait matrix removal was recorded at regular intervals. For the first week of the test, each jar was examined daily for termite removal of bait, and categorized by the percentages of bait removed (Table 1). This provided an in-depth view of the activity on baits during their initial discovery and acceptance (or rejection) by the termites. To provide bait material for a longer test, on the 7th day of the test, 0.5 g of bait was added to the amount left in each replicate, and bait removal values were reset to 'none.' On day 21 at the half-way point of the test, 5ml of distilled water was added to all surviving replicates. During the last 5 weeks of the test, data were collected weekly. Other notes and observations, such as percentage sand cover of the bait and presence of dead individuals in the arena were also recorded on a regular basis. Jars were examined in this manner for a total of 6 weeks (42 days). As replicates reached 100% termite mortality, they were disassembled, counting surviving termites, making final estimations of bait removal, and cleaning *P. ponderosa* blocks for drying and mass loss determination. All replicates surviving to 6 weeks were disassembled on the 42nd day.

Two-choice test statistical analysis

Following final data collection for all replicates, mortality data and

Table 1. Scoring system and daily estimation of bait removal by subterranean termites.

Score	Estimated Bait Removal (%)
No	0
Little	10
Some	25
Moderate	50
High	75
Nearly Complete	90
Complete	100

wood mass loss data (as percentages) were arcsin-square root transformed to normalize the data sets. The transformed data were subjected to separate analyses of variance (ANOVA; SAS Institute 1999). Mean separations were performed with the Ryan-Einot-Gabriel-Welsch Multiple Range (Q) test (REGWQ; SAS Institute 1999), using an α of 0.05. Percentage bait removal were subjected to general linear model time series analysis (GLM; SAS Institute 1999), with means separation using REGWQ. The first week (daily) bait removal data were analyzed separately from the data for the remaining weeks.

RESULTS

Multiple choice arena

Mean (\pm SEM) initial dry masses of the three baits were 0.2007 ± 0.0002 g for bait 1, 0.1010 ± 0.0002 g for bait 2, and 0.2409 ± 0.0002 g for bait 3 ($n=15$ for each). Mean post-test dry masses were 0.1525 ± 0.0171 g for bait 1, 0.0905 ± 0.0049 g for bait 2, and 0.0656 ± 0.0183 g for bait 3. Kruskal-Wallis results of the actual percentage bait mass loss indicated a significant difference between at least one of the two possible bait pairs ($df = 2$; $H = 19.43$; $P < 0.001$). Median (Q_1 , Q_3) percentage bait losses for each bait were 8.7 (6.84, 10.3)% for bait 1, 5.7 (4.61, 6.61)% for bait 2, and 88.99 (69.62, 90.61)% for bait 3. IQR's did not overlap for any of the three baits. Thus, bait 3 was removed significantly more than bait 1, which was removed significantly more than bait 2. For estimated percentage mass loss, a significant difference between at least two pairs was indicated (adjusted for ties: $df = 2$; $H = 16.19$; $P < 0.001$). Median (Q_1 , Q_3) estimated percentage bait losses for each bait were 10.0 (0.0, 10.0)% for bait 1, 0.0 (0.0, 0.0)% for bait 2, and 50.0 (10.0, 90.0)% for bait 3. The trend observed in the estimates appears in the mass loss data as well: mass loss was bait 3>bait 1>bait 2. Mass loss was significantly correlated with estimated bait removal for the three baits combined ($r = 0.694$; $df = 1, 44$; $P < 0.0001$; Fig. 3).

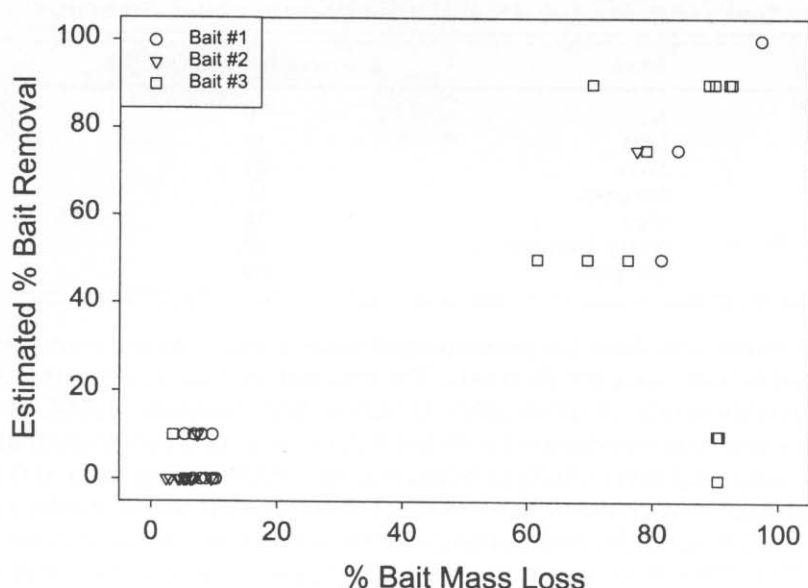


Fig. 3. Estimates of the percentage bait mass loss data plotted against actual percentage bait mass loss in the multiple choice bait acceptance study (Spearman's rank-order correlation, $r = 0.694$; $df=1.44$; $P<0.0001$).

Two-choice test mortality

Percentage survival of replicates at each time period, is presented graphically in Fig. 4. Both DOT baits exhibited an apparently linear decrease in survivorship over time, starting at day 21 for 2% DOT and day 28 for 1% DOT. Both zinc borate rates were unable to completely remove a replicate from the test, even on the final day, similar to the blank bait. Mean percentage mortality data at the end of the experiment ranged from $10.6 \pm 2.6\%$ for controls to $100.0 \pm 0.0\%$ for 2% DOT laden bait (Table 2). Both DOT baits were associated with significantly greater mortality than either of the ZB baits, which in turn did not differ significantly from the controls ($df = 4, 24$; $F = 311.12$; $P < 0.0001$; Table 2). However, with both toxicants, the rate of AI in the bait matrices did not contribute significantly to mortality (Table 2).

Two-choice test wood mass loss

Percentage wood mass loss ranged from $9.00 \pm 4.5\%$ for 2% DOT bait to $67.63 \pm 4.5\%$ for 2% ZB (Table 2). Both DOT baits were associated with significantly lower percentage wood mass loss than either of the ZB baits and the control ($df = 4, 24$; $F = 42.36$; $P < 0.0001$; Table 2). Blank bait (control) percentage wood mass loss did not differ significantly from those with either ZB bait (Table 2). As with the mortality data, bait

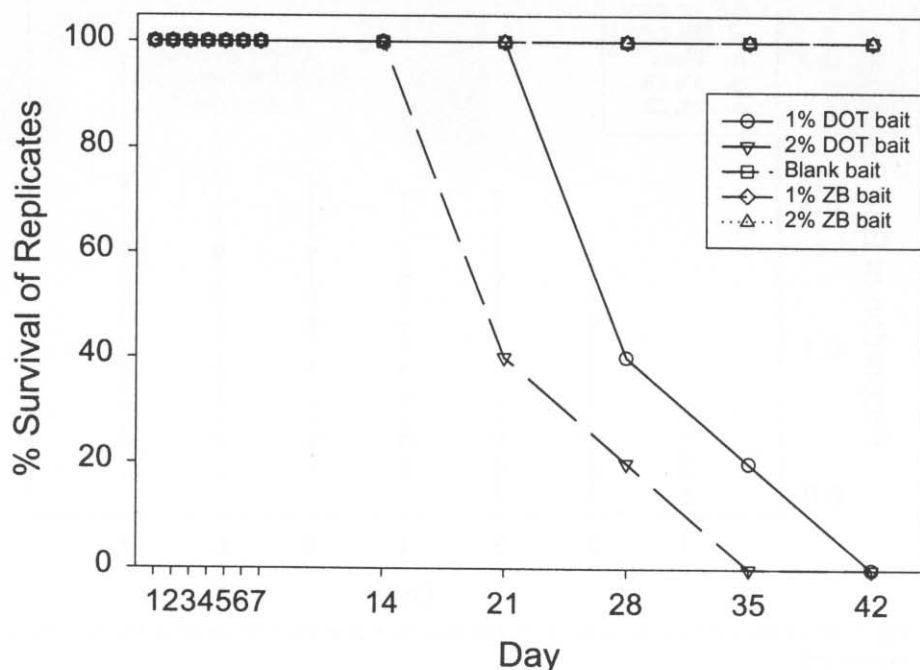


Fig. 4. Percentage survival of replicates of *C. formosanus* in paired choice tests of *P. ponderosa* blocks vs a variety of boron-containing bait matrices.

percentage AI did not influence wood mass loss significantly (Table 2).

Two-choice test bait removal

Bait removal over time is presented for the first week of daily observations in Fig. 5. The entire data set (daily and weekly observations) is presented in Fig. 6. During the first week, there were significant differences among treatments ($df = 4, 24$; $F = 4.92$; $P = 0.001$), as well as an effect of time (as day; $df = 6, 24$; $F = 5.37$; $P < 0.0001$); however, there was no time \times treatment effect ($df = 4, 24$; $F = 0.56$; $P = 0.95$). The

Table 2. Mean mortality and mass loss of untreated wood wafers (as %; \pm SEM) by bait treatment.

Treatment	n	Mortality	REGWQ [†]	Wood Mass Loss	REGWQ [†]
1% DOT	5	99.90 \pm 0.06	a	10.32 \pm 4.1	b
2% DOT	5	100.00 \pm 0.0	a	9.00 \pm 4.5	b
Blank	5	10.60 \pm 3.2	b	49.67 \pm 0.9	a
1% ZB	5	14.50 \pm 2.6	b	65.93 \pm 2.6	a
2% ZB	5	16.95 \pm 4.0	b	67.63 \pm 4.5	a

[†]Values having the same letter within columns are not considered significantly different at the $\alpha=0.05$ level by REGWQ.

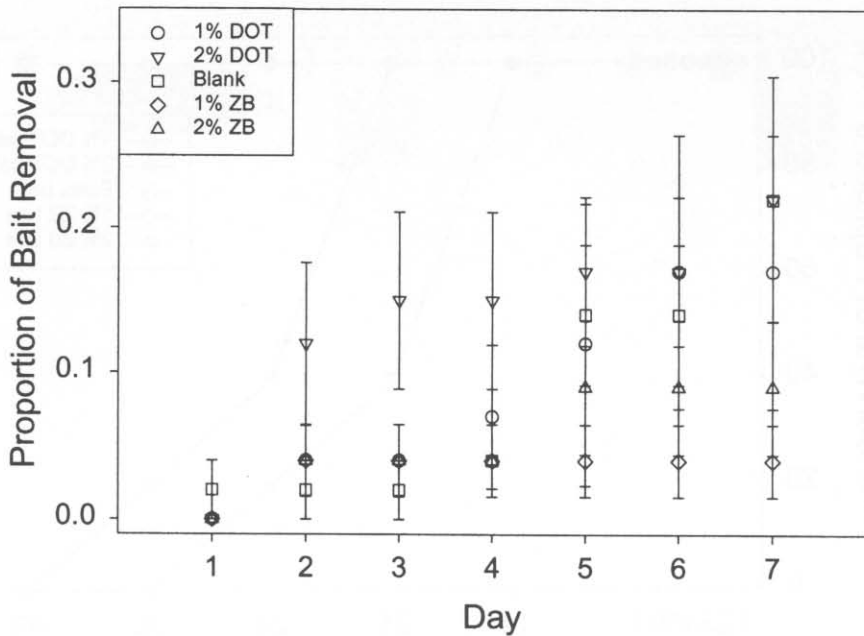


Fig. 5. Proportion of bait removal by *C. formosanus* during the first week of the two-choice experiment.

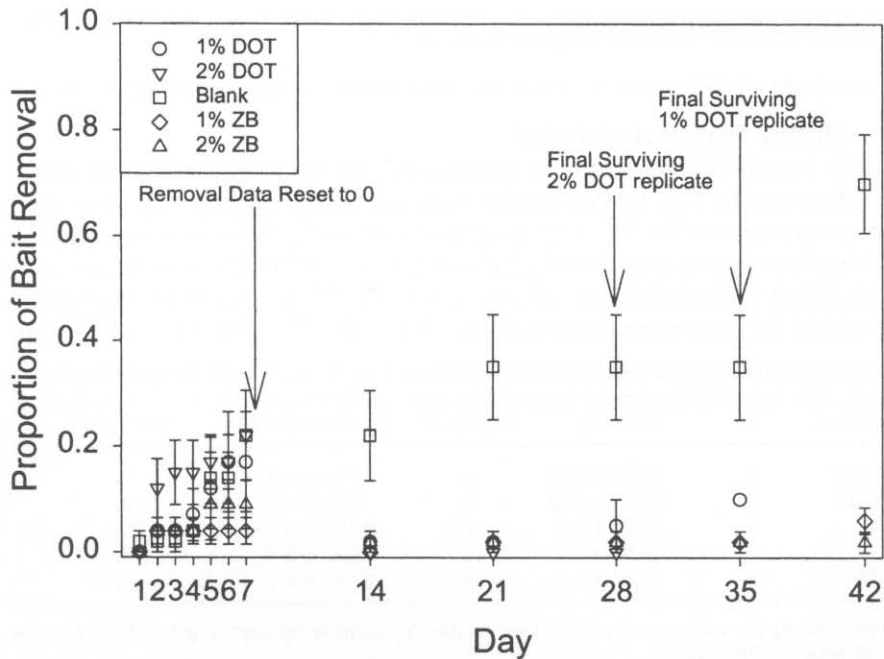


Fig. 6. Proportion of bait removal by *C. formosanus* during the full 6 weeks of the two-choice experiment.

2% DOT bait was removed significantly more than all other baits during the first week, while blank bait and the 1% DOT baits were removed equivalently but in significantly greater amounts than either ZB bait. Day 1 had significantly lower removal than day 7, while the other days overlapped greatly.

Removal data for the remaining weeks indicated a significant difference among treatments ($df = 4, 16$; $F = 69.3$; $P < 0.0001$), day ($df = 4, 16$; $F = 3.94$; $P = 0.005$), and time \times treatment interaction ($df = 4, 16$; $F = 2.99$; $P = 0.0004$). Blank bait was removed significantly more than all other bait treatments. Day 42 showed significantly greater bait removal than all other days.

Percentage sand cover of the baits showed promise for assessing differences in bait acceptance by *C. formosanus*. Mean proportion (\pm SEM) of bait sand coverage appears graphically in Fig. 7. While all baits received some degree of sand cover ($\sim 5\%$), sand coverage was highest for the ZB baits (Fig. 7). It should be noted, however, that one replicate of blank bait became entirely covered with sand by the end of the experiment. Upon removal of the sand during disassembly of this

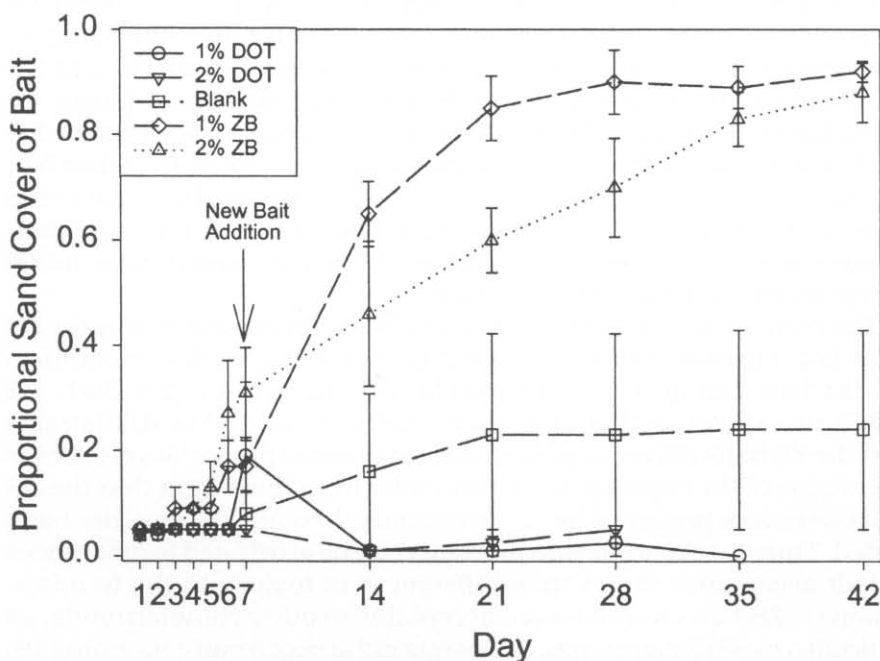


Fig. 7. Proportion of baits covered with sand by *C. formosanus* during the full 6 weeks of the two-choice experiment.

replicate's arena, we found that this bait had been entirely removed by the termites beneath the sand.

DISCUSSION

In our multiple choice assay, we tested the validity of using estimated mass loss data in place of actual mass loss data over a short-term trial. While short (24 hr) trials might not actually necessitate using estimated loss when the actual mass loss can be measured over that single time period, such periodic estimates could be very useful in longer term tests, as shown in our borate bait trials. At 24 hrs, estimated percentage bait removal was correlated with the actual percentage mass loss data. Thus, these data indicate that observational estimates of bait removal can be valid for comparative purposes. However, care must be taken when making these measurements to ensure consistency.

By using the two-choice method we differentiated between two bait toxicants, at two rates each. Observational data on bait removal demonstrated differences between acceptability of these baits that was not apparent from final termite mortality or wood mass loss. Although both wood mass loss and termite mortality indicated that the DOT baits were superior to the ZB baits, no significant differences existed for determining which DOT rate was more effective in controlling *C. formosanus*. Survival of replicates (Fig. 4) indicated that 1% and 2% DOT baits were equally effective, but 1% took one week longer for mortality to commence. However, bait removal data indicate that the 2% DOT bait was actually removed significantly more than any other bait during the first week of the experiment. Thus, the 2% DOT bait could be considered superior to the non-DOT baits in this study as demonstrated by both greater (and faster) mortality rate, and greater initial acceptability than the non-DOT baits.

The removal data over time also gives us an impression of why the ZB baits failed to elicit significant mortality. In the first week of encountering the bait, termites removed significantly more of the 2% DOT, 1% DOT and control bait than either of the ZB baits tested. Fig. 6 illustrates that the ZB baits did not approach a mean removal proportion of 0.2 over the course of the experiment. We can infer from these data that the ZB baits were less preferred by *C. formosanus* than any of the other baits tested. Thus the differences in mortality can be attributed to differences in bait acceptance rather than differences in toxicity to the termites. However, ZB baits may be more acceptable to other rhinotermitids, as *Reticulitermes* Holmgren spp. in Georgia did accept a bait containing 1% ZB in a powdery cellulose matrix (Forschler 1996).

Boron compounds have been examined for use in baits for other species of subterranean termites, such as *Heterotermes aureus* (Snyder) (Jones 1991), *Reticulitermes flavipes* (Kollar) (Grace 1990; Grace *et al.* 1990; Forschler 1996), and *R. virginicus* (Banks) (Forschler 1996). Grace (1990) estimated that barium metaborate monohydrate at a rate of 0.5% (5,000 ppm) by weight might make an effective bait toxicant for *R. flavipes*. For both *H. aureus* (Jones 1991) and *R. flavipes* (Grace *et al.* 1990), avoidance of materials containing greater than 5,000 ppm (or 0.5%) DOT were observed. In forced feeding assays using *C. formosanus*, 100% mortality was achieved with pressure treated Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] blocks containing as little as 0.29% DOT (0.35% boric acid equivalents; BAE) by Grace *et al.* (1992) within 3 weeks. Grace & Yamamoto (1994) provided evidence that DOT concentrations of 1% or greater were effective at reducing *C. formosanus* attack on treated wooden (*P. menziesii*) boards. Our data indicate that *C. formosanus* will remove up to 2% DOT impregnated bait at least during the first week of exposure. However, either due to increasing mortality or learned (social) avoidance, bait removal in subsequent weeks was dramatically lower than that of the first week (Figs. 5 & 6).

Sand cover of baits can provide some measure of bait acceptance by termites. Comparing the data in Figs. 6 & 7, a picture of bait acceptance (or rejection) of these baits is apparent. Those baits with generally low acceptability (*i.e.*, low removal) by the termites, were covered with sand; whereas more acceptable baits were not covered with as much sand. However, exceptions to this rule did occur, as in the one control replicate where the bait was covered entirely and consumed from beneath the sand. Thus, sand cover alone is not a definitive indicator of the relative acceptability of a food source.

The arenas and the data collection strategies described in this paper are of value for assaying bait matrices. Observational data can provide additional information on the performance of baits beyond the standard assay information on wood or bait mass loss and termite mortality. However, consistency is very important. The same individual should perform the entire experiment, from the initial placement of the bait matrix to the end estimation of the total bait consumption. While estimation of any sort is fraught with subjective considerations, consistent personnel can reduce this source of error. We believe that observational data are particularly useful for evaluating initial bait acceptability in laboratory trials, where the short time period and the texture of the bait matrix may make it very difficult to record any more quantitative data.

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