Bait Matrix for Delivery of Chitin Synthesis Inhibitors to the Formosan Subterranean Termite (Isoptera: Rhinotermitidae)

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J. Econ. Entomol. 94(2): 506–510 (2001)

ABSTRACT The efficacy of three chitin synthesis inhibitors, diflubenzuron, hexaflumuron, and chlorfluazuron, incorporated into a novel bait matrix to kill the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, was evaluated in the laboratory. The bait matrix was significantly preferred by *C. formosanus* over southern yellow pine wood in a two-choice feeding test. Bait formulations containing 250 ppm of the three chitin synthesis inhibitors were presented to termite nests with 2,500 individuals (80% workers and 20% soldiers) in the presence of alternative food sources consisting of cardboard and southern yellow pine, *Pinus taeda* L., wood. None of the bait formulations were significantly repellent or feeding deterrent to the termite workers evidenced by the lack of full consumption of alternative food sources. All nests presented with the bait formulations died within 9 wk, whereas the control nests (bait with no chitin synthesis inhibitors) remained alive 6 mo after the end of the study. No significant differences in consumption were observed among the chitin synthesis inhibitor treatments. Importance of this study for the improvement of current bait technology is discussed.

KEY WORDS subterranean termite, bait, chitin synthesis inhibitors, control

THE FORMOSAN SUBTERRANEAN termite, *Coptotermes formosanus* Shiraki, native to China, was introduced into the continental United States during the late 1960s (Beal 1987). Since then, it has caused millions of dollars in damage (Su and Tamashiro 1987, Su and Scheffrahn 1990). Wooden structures and living trees in New Orleans, LA, have been severely damaged by this pest (LaFage 1987, Rojas et al. 2001).

Different approaches have been used to try to control subterranean termites. Among the newest and more effective is the use of low-toxicity baits containing growth regulators (Su 1991, Su and Scheffrahn 1993, Shaheen 1997). Termite baits deliver toxicants via ingestion, and their effectiveness is dependent upon the willingness of termites to consume the bait when presented with choices of other cellulose food sources (Grace et al. 1996, Henderson and Forschler 1996). Optimal foraging theory postulates that animals are able to choose their food according to nutritional needs (Emlen 1973) to optimize fitness (Krebs 1978). By presenting a bait toxicant to an insect within an optimal food source, the probability of consumption of the toxicant could be increased. As a means to improve toxicant consumption by Formosan termites, a nutritionally based bait matrix was developed based on research we have conducted with the Formosan subterranean termite (M.G.R., unpublished data). The ingredients in the matrix were chosen based on nutritional value to the Formosan termite. The first objective of this research work was to determine whether a nutritionally enhanced bait matrix would be more preferred by the Formosan termite than southern yellow pine, *Pinus taeda* L., wood (the most abundant wood type available based on building construction in the southern United States). The second objective was to test the feasibility of using a nutritionally enhanced matrix as a substrate to deliver chitin synthesis inhibitors to the Formosan subterranean termite without reducing consumption or preference.

Materials and Methods

Biological Material. Formosan subterranean termites for this study were collected from three different localities around the New Orleans metro area. Colonies were collected from three types of material: (1) an infested pine (*Pinus* sp.) log, (2) carton (material produced by termites to built nest structures) from a house wall, and (3) mulch from trees in a New Orleans city park dump. All the termites were brought to the laboratory in plastic containers. The infested pine log was transferred to a 75.7-liter plastic trash container, containing 10 liters of topsoil: sand mixture at 1:1 ratio and 3 liters of distilled water. Termites from the carton material and the mulch were harvested by breaking apart the substrate and shaking the termites directly onto or through a sifter onto paper towels. Once the termites were separated from the debris, groups of ≈10,000 termites were transferred to rectangular 11.4-liter Sterilite plastic boxes (42 by 29 by 15 cm) containing 4 liters of the above mentioned soil-sand-water mixture. Different types of wood were added as food and the containers were covered with

Table 1. Bait matrix formulation

Ingredient	Amount, g	Product information
Cellulose	250.00	Bio-Serv #3425
Distilled water	5.62	
Drinking water	750.00	Barbe's Dairy, West Wego, LA
Ethyl alcohol	3.75	Quantum MT #194A31
Ergosterol	0.45	Sigma #E-6510
Lecithin	1.25	USB #18240
Water-storing polymer[a]	0.187	Terra-Wet T-400, San Diego, CA
Yeast hydrolyzate	1.50	ICN Biomed. #103304

[a] Cross-linked potassium polyacrylate/polyacrylamide copolymer.

lids and maintained in complete dark at 27 ± 3°C. To ensure that the termites were not traumatized by the collection and sifting process, boxes containing termites were left undisturbed for at least 1 wk before preparation of the experimental units. A period of nondisturbance was included because prior experience has shown that termites respond poorly to food after handling.

Bait Matrix Formulation. The bait matrix was formulated to closely resemble the chemical composition of highly preferred wood species infected by wood-decaying fungi. The wood species used were yellow birch, *Betulla alleghaniensis* Britton; red gum, *Liquidambar styraciflua* L.; and pecan, *Carya illinoensis* (Wangenh.), which were significantly more preferred by the Formosan termite than most other commercially available wood species (Morales-Ramos and Rojas 2001). Blocks of these wood species were infected with the wood decaying fungus *Curvularia lunata* (Wakker), reported to be associated with the Formosan termite (Rojas et al. 2001), and incubated at 27°C for a 1-mo period. The infected wood blocks were chemically analyzed for their sugar, free amino-acid, fatty acid, and esterol content using high performance liquid chromatography and gas chromatography (M.G.R., unpublished data).

The list of ingredients of the bait matrix is presented in Table 1. The matrix was composed of 75% water, 24.69% cellulose, and 0.31% nutritional supplements and additives. A water storing polymer (cross-linked potassium polyacrylate/polyacrylamide copolymer, T-400; Terawet, Sand Diego, CA) was added to retain water within the matrix.

The bait matrix was prepared by mixing lecithin, ergosterol, ethyl alcohol, and Barbe's spring water into a 1-liter glass bottle by using a glass rod. The opening of the bottle was covered with a foam stopper and the stopper covered with foil and autoclaved at 120°C for 20 min. After autoclaving, the bottle was closed tightly with screw caps and allowed to cool to room temperature. The cellulose was weighed into a 1-liter glass beaker covered with foil and autoclaved as described above. The water-storing polymer was weighed into a 125-ml glass beaker, distilled water was added, and the beaker was tightly covered with foil and autoclaved. After autoclaving, the beakers were allowed to cool to room temperature.

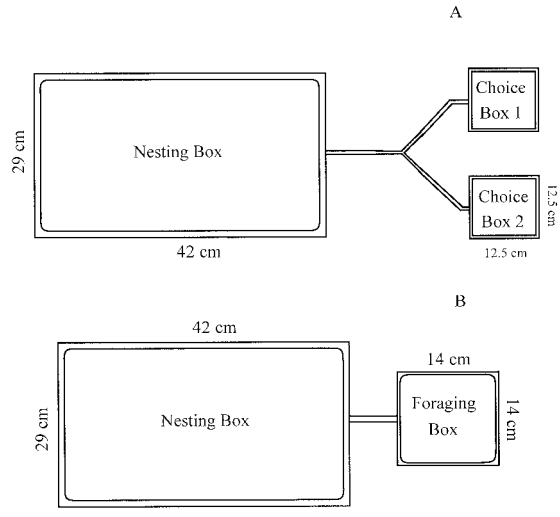

Fig. 1. Schematics of experimental test boxes. (A) Two-choice test. (B) Foraging box.

Under a laminar flow hood, the yeast hydrolysate was added to the lecithin-containing mixture by using a sterile spatula. The mixture was shaken until the yeast hydrolysate was incorporated. Using a sterile spatula, the lecithin-containing mixture was added to the cellulose and mixed well by using an electric mixer (Ultra Power, KitchenAid, St. Joseph, MI). Finally, the water-storing polymer was added to the mixture and homogenized. The container was covered with a sterile paper towel and tightly covered with a plastic lid to prevent microbial contamination and evaporation.

Bait Matrix Preference. A two-choice test was used to determine whether the bait matrix was preferred over southern yellow pine by the Formosan termites.

A choice unit consisted of three plastic containers connected to each other by 6.25-mm-diameter transparent flexible PVC tubing (Nalgene 8000-9060, Nalgene Nunc International, Rochester, NY), one nesting box, and two choice boxes (Fig. 1). Nesting boxes were filled with 4 liters of the above-described mixture of sand-soil:water. A hole was drilled into the side of each nesting box and a 10-cm length of the transparent flexible PVC tubing was attached at the opening. The opposite end of this piece of tubing was attached to a plastic Y connector. Two, 10-cm lengths of tubing were attached to the two other openings in the Y connector. The opposite end of each of these pieces of tubing was attached to a separate smaller plastic choice box 12.5 by 12.5 by 4 cm (591-ml capacity) containing 80 ml of a 1:1 top soil: sand mixture (Fig. 1A).

Bait matrix units consisted of a combination of bait matrix and a protective outer layer of paraffin. Bait matrix units were formed by dipping 25-g pieces of bait matrix in paraffin (Fisher #P31-500, Fisher, Pittsburgh, PA) at 70°C. These units were allowed to cool to room temperature. Each unit was then weighed to establish the weight of the paraffin coating on each unit. The pine wood was dried in a vacuum oven at

58°C and 50.8 cm of mercury (20 inches, Hg) for 24 h and exposed to a 96% RH atmosphere for 1 h before weighing the samples.

One 25-g bait matrix unit was placed in one of the two choice boxes of a choice unit. Two pieces of southern yellow pine wood (1.6 by 1.6 by 12.5 cm) with a combined dry weight of ≈25 g were placed in the other choice box of the same choice unit.

Five different groups of termites of varying sizes (3,000–5,000 individuals) were drawn from five different colonies and placed in nesting boxes. Bait matrix units and pine wood samples were exposed to each group of termites for 8 d during the first replication to establish consumption rates. Consumption rates indicated that group 5 was larger than the rest and the exposure time of food samples was adjusted accordingly. Groups 1–4 were exposed to the samples for 27 d and group 5 for 17 d. The test was replicated six times in groups 1–4 and 11 times in group 5. After exposure to the termites, the matrix units were cleaned of soil and termites and weighed again. The previously established paraffin weight was subtracted from the final weight of matrix units. Pine wood samples were cleaned, dried in the vacuum oven for 24 h, exposed to 96% RH for 1 h, and weighed. Consumption rates were calculated by dividing total weight consumed (in milligrams) by the number of days of exposure yielding milligrams per day. The weight of water contained in the matrix (75% Table 1) was subtracted from the consumed weight to obtain dry weight consumption of bait matrix. The means of daily consumption rates of bait matrix and pine wood were statistically compared by analysis of variance (ANOVA) and Student's *t*-test.

Chitin Synthesis Inhibitors. Three technical grade (99%) chitin synthesis inhibitors, diflubenzuron, chlorfluazuron (Ensystex, Fayetteville, NC), and hexaflumuron (#PS-2079; Chemical Service, West Chester, PA) were chosen for this test. The rationale for choosing these three chemicals was based on the results obtained by Su and Scheffrahn (1993) indicating that hexaflumuron was nonrepellent to Formosan termites, whereas diflubenzuron induced significant repellence to this termite species. Additionally, chlorfluazuron was chosen as its use against other insect species had shown it to be an effective chitin synthesis inhibitor (Elek 1998).

Experimental units to test matrix-chitin synthesis inhibitor combinations consisted of nesting boxes connected to foraging boxes by flexible PVC tubing described above (Fig. 1B). Nesting boxes consisted of Sterilite plastic boxes (42 by 29 by 15 cm) containing 3 liters of the top soil:sand mix (1:1), 3 g of water-storing polymer, 800 ml of distilled water, and 16 g of cardboard. Approximately 2,500 termite at a ratio of 80% workers and 20% soldiers were placed into each nesting box. The foraging containers were constructed from 946-ml self-sealing plastic boxes (14 by 14 by 7 cm) filled with 500 g of sand with water, at a 3:1 ratio, and 100 mg of the water-absorbing polymer (Fig. 1B). The foraging boxes were provided with 50 g of bait and 14 g of pine wood. Nine experimental units per treatment and nine controls (replications) were used.

The three chitin synthesis inhibitors were separately incorporated in the bait matrix at concentrations of 250 ppm (concentration of active ingredients in dry weight of matrix equivalent to ≈1,000 ppm). Preliminary results testing a range of chitin synthesis inhibitor doses from 50 to 5,000 ppm, showed that 250 ppm was sufficient to induce mortality within a period not significantly different than that of higher doses. A total of 100 mg of each chitin synthesis inhibitor was weighed using a Mettler balance (model PB303; Fisher) and placed into a 50-ml sterile screw cap conical tube (#62.547.004; Sarstedt, Newton, NC) and dissolved with 1-ml acetone (#9006-03; J. T. Baker, Phillipsburg, NJ). Under a laminar flow hood, the CSI solution was mixed with 40 ml of sterile nutritional supplement (ingredients in Table 1 except cellulose) prepared as described above. The tube was tightly closed with a screw cap and manually shaken for 30 s. The mixture was added to 100 g of sterile cellulose contained in a 600-ml sterile glass beaker. The tube was then rinsed three times with 40-ml of nutritional supplement and added to the cellulose. Additional 260 ml of nutritional supplement was added to make total volume of 300-ml of supplement in 100 g of cellulose. The mixture was manually homogenized using a stainless steel spatula.

To encase the bait matrix, tubes made of fibrous casing material (#124B; L.E.M. Products, Miamitown, OH) were cut into 150-mm lengths. Each piece was turned inside out and one of the ends of the tube was tightly tied with a rubber band to form a pouch. Tubes were turned inside out because termites could more easily penetrate this particular casing when presented with the interior wall. Fifty grams of bait matrix containing a single chitin synthesis inhibitor was compacted into one pouch at which point the open end of the tube also was closed with a rubber band (Rojas et al. 1999). The bait casing was placed inside of the foraging box adjacent to a 10-g piece of pine wood, taking care that they were partially covered with the sand. Control bait matrix was prepared minus any chitin synthesis inhibitor and presented in the same way as the treated matrices. To eliminate variability in the time of discovery of the food source, 20 termites from each nesting container were transferred to the corresponding foraging container box to initiate foraging as soon as possible. The termites placed in the foraging box were expected to tunnel back to the nesting box, creating connecting tunnels (possibly with trail pheromone) between the boxes.

All the experimental boxes were maintained under dark at 29 ± 2°C, and 75 ± 2% RH. Observations were done every 48 h until all the termites died. After 2 wk of exposure, the bait casing was removed from the foraging box and bait consumption was measured. Consumption was measured by weighing the remaining bait with the Mettler balance and subtracting the results from the initial bait weight. Replacement bait casings containing the same chitin synthesis inhibitor were placed in the foraging boxes as termites con-

Table 2. Consumption rates in milligrams per day of a nutritionally based bait matrix (dry weight) and blocks of Southern yellow pine wood by five groups of 2,000–5,000 Formosan subterranean termites

Group no.	Consumption rates[a]		t^a	df	P
	Bait matrix	Pine wood			
1	254.9 ± 145.5	21.5 ± 14.7	3.8	10	0.0033
2	109.9 ± 61.9	15.1 ± 14.0	3.8	10	0.0037
3	199.2 ± 71.9	17.7 ± 18.6	5.9	10	0.0001
4	97.5 ± 65.4	22.3 ± 16.4	2.9	10	0.0147
5	434.1 ± 161.8	85.2 ± 97.5	6.8	20	<0.0001

[a] In milligrams per day; mean ± SD.
[b] Student's t values higher than 1.812 indicate significant differences between means ($\alpha = 0.05$).

sumed the bait. The time to reach 100% mortality was measured and recorded. Mean comparisons among treatments and control were conducted. Bait consumption was statistically compared by ANOVA and mean differences between the treatments were compared using the Tukey–Kramer honestly significant difference (HSD) test at a = 0.05.

Results

Bait Matrix Preference. The difference between mean consumption rates (in milligrams per day) of bait matrix and southern yellow pine was highly significant. Formosan subterranean termites consumed significantly more bait matrix (249.73 ± 178.25 mg/d dry weight) than southern yellow pine wood (32.16 ± 50.75 mg/d dry weight) ($t = 6.9$, df = 68, $P < 0.0001$) in the overall analysis. Comparisons within groups showed significantly higher consumption rates of bait matrix than southern yellow pine wood in all five groups (Table 2).

Chitin Synthesis Inhibitors. None of the chitin synthesis inhibitor treatments induced repellence or feeding deterrence to the Formosan termites evidenced by the lack of consumption of pine wood and cardboard provided as alternative food sources. The mean bait consumption after 2 wk was not significantly different among chitin synthesis inhibitor treatments (10.99 ± 2.2, 11.53 ± 2.2, and 11.39 ± 1.5 g in hexaflumuron, diflubenzuron, and chlorfluazuron, respectively). However, the control group consumed significantly more bait (14.43 ± 2.5) than the chitin synthesis inhibitor treatments ($F = 4.89$; df = 3, 32; $P = 0.0065$) (Table 3).

The chitin inhibitors achieve 100% mortality within 9 wk, with no significant difference among the treatments (Table 4). Control colonies remained alive after the end of the test and were still living 6 mo after. High variability was noted in the time required to reach 100% mortality; this effect was attributed to the age structure of the termite groups, the groups composed of mixed instars died sooner than the colonies composed of fourth instars only.

Table 3. Bait consumption in grams of termite nests 2 wk after exposure to 50 g of bait matrix with alternative food sources consisting of 16 g of cardboard and 10 g of pine wood

Treatment	Consumption
Control	14.43 ± 2.51a
Diflubenzuron	11.53 ± 2.20b
Chlorfluazuron	11.39 ± 1.53b
Hexaflumuron	10.99 ± 2.22b

Mean ± SD, means with the same letter are not significantly different after ANOVA Tukey–Kramer HSD test ($\alpha = 0.05$; $F = 4.89$; df 3, 32; $P = 0.0065$), $n = 9$.

Discussion

Formosan termites consumed significantly more of the nutritionally based matrix (7.8 times as much dry weight) than southern yellow pine wood in the two-choice foraging test. The feeding preference for the nutritionally based bait matrix over Southern yellow pine by the Formosan termites could be highly advantageous in the field where baits compete with abundant sources of yellow pine (mainly houses), especially in the Southeastern United States.

None of the three chitin synthesis inhibitors tested induced any significant repellence or feeding deterrence to the Formosan termites. Repellence is defined as stimuli that cause oriented movements away from the source, and feeding deterrence as stimuli that inhibits feeding (Matthews and Matthews 1978). These results contrast with those reported by Su and Scheffrahn (1993) where concentrations of diflubenzuron smaller than 2 ppm (dry weight of pine blocks) were found to be repellent to the Formosan termite. There were two important differences between the work of Su and Scheffrahn (1993) and our work: (1) the use of pressure-impregnated pine blocks by Su and Scheffrahn (1993) versus our use of a nutritionally based bait matrix, and (2) differences in the purity of the diflubenzuron used in each study.

Su and Scheffrahn (1993) used a pressure impregnation method that may have resulted in concentrations of active ingredient on the outside of the pine blocks that were higher than the stated concentrations as hypothesized by Su in a subsequent article (Su and Scheffrahn 1996). The diflubenzuron used by Su was 95% pure. The purity of the diflubenzuron used here was 99%.

Another explanation may be that feeding preference for the bait matrix may overcome any potential

Table 4. Survival of *C. formosanus* colonies presented with three different chitin synthesis inhibitors and control

Weeks	No. of colonies alive			
	Control	Hexaflumuron	Diflubenzuron	Chlorfluazuron
0–3	9	9	9	9
4	9	7	8	9
5	9	6	6	5
6	9	6	5	4
7	9	4	3	2
8	9	3	3	1
9	9	0	0	0

feeding deterrence induced by the chitin synthesis inhibitors. The nutritionally based bait matrix used in this study may be so highly preferred by Formosan termites as to negate any feeding deterrence induced by the chitin synthesis inhibitors. Also, pine wood is not a highly preferred wood species by Formosan termites (Morales-Ramos and Rojas 2001).

Termites consumed significantly more bait in the control group than in all the chitin synthesis inhibitor treatments. Lower consumption by termites feeding on the treated matrices was most likely the result of the effect of the chitin synthesis inhibitors after the termites ingested them with consumption declining as worker mortality increased. Such a decline was evident among groups ingesting chlorfluazuron within approximately 3 wk of the initiation of feeding and within 4 wk for the groups consuming diflubenzuron and hexaflumuron. This difference in consumption cannot be attributed to feeding deterrence because termites ignored alternative food sources, consisting of cardboard and pine wood, in favor of the bait in all treatments.

All chitin synthesis inhibitors achieved 100% mortality of termite colonies within 9 wk, with no significant difference among the treatments. The matrix tested in this study was shown to be effective as a carrier of chitin inhibitors such as diflubenzuron, hexaflumuron, and chlorfluazuron.

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Received for publication 4 October 2000; accepted 9 December 2000.