EFFECT OF A DECAY FUNGUS ON SUBTERRANEAN TERMITE (ISOPTERA: RHINOTERMITIDAE) RESPONSE TO BAIT TOXICANT TREATED WOOD[1]

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Abstract—In no-choice feeding tests, *Reticulitermes flavipes* (Kollar) weekly survival and feeding on two bait toxicants was compared for sound sweetgum blocks and those decayed by *Gloeophyllum trabeum* (Pers. ex Fr.) Murr. At 100 ppm, imidacloprid and sulfluramid showed promise for subterranean termite control. Termite survival was significantly reduced within 7 days. After 28 days, all termites were dead in tests with imidacloprid, and only a few survived in tests with sulfluramid. Termites in the untreated controls fed significantly more on decayed blocks than on sound wood at all time intervals. Despite the termites' slight preference for treated decayed wood rather than treated sound wood, mortality ensued quickly after exposure to either substrate. This lack of dramatic difference in wood consumption and mortality suggests that the time-consuming and labor-intensive process of decaying blocks is not justified for either bait toxicant at 100 ppm in wood. Despite low termite survival in the treatments, significant differences in block weight loss occurred over time. Wood degradation may have resulted from mould fungi emanating from the large numbers of dead and decaying termites associated with the treatments. This study also demonstrates the importance of correcting for wood weight loss attributable to experimental conditions, particularly when using decayed wood.

INTRODUCTION

A complex relationship exists between termites and fungi (reviewed by Amburgey, 1979). Termites preferentially consume wood decayed by certain fungi rather than sound wood, but they avoid wood decayed by other fungi. Esenther et al. (1961) reported that termites were apparently attracted to wood decayed by the brown-rot fungus *Gloeophyllum trabeum* (Pers. ex Fr.) Murr. (formerly *Lenzites trabea* Pers. ex Fr.). Subsequent research has shown that many Isoptera show a positive orientation to the decayed sawdust (Gao, 1987) or extracts of this fungus (Allen et al., 1964; Matsuo and Nishimoto, 1974; Smythe et al., 1965, 1967a,b). Many termite species also consume greater quantities of decayed wood than sound wood (French et al., 1981; Lenz et al., 1980; Matsuo and Nishimoto, 1973; Ruyooka, 1978/1979; Smythe et al., 1971), although Grace et al. (1992) reported that *G. trabeum* inhibited feeding by the Formosan subterranean termite.

Esenther et al. (1961) suggested that decayed wood might be used in termite control strategies. This suggestion led to the bait-block method of termite control (Esenther and Coppel, 1964; reviewed by Esenther and Beal, 1979), which proposed that a termite colony could be suppressed or even eliminated if foraging termites fed on a slow-acting chemical incorporated into a small block of decayed wood.

In the earliest field trials to evaluate a bait toxicant, *Reticulitermes* spp. fed on mirex-treated decayed wood, and the colonies apparently were suppressed (Beard, 1974; Esenther and Beal, 1974, 1978; Esenther and Gray, 1968; Ostaff and Gray, 1975). It is not known whether termites would have accepted undecayed blocks treated with these mirex concentrations (4,000 ppm). Indeed, Su and Scheffrahn (1991) indicate that such high concentrations deter feeding by *R. flavipes* (Kollar) in the laboratory, and they suggest that "the use of decayed wood might have masked the deterrent concentrations of mirex." Mirex was no longer investigated as a bait toxicant in the United States after its use was phased out and then banned by the Environmental Protection Agency during the mid-1970's (Kaiser, 1978). Mirex continues to show promise as a bait toxicant in Australia (French, 1988, 1991; Paton and Miller, 1980) and in China (Gao, 1987), where it generally is incorporated into a substrate that includes decay fungi.

In the past decade, U.S. researchers have shown renewed interest in evaluating chemicals for use in the bait-block method. This technique offers a potential alternative to the current practice of

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applying large quantities of a persistent chemical to soil beneath a structure to create a barrier to subterranean termite movement. Although the original concept was to use the fungus in conjunction with a slow-acting chemical, a treated, decayed cellulosic substrate has seldom been used, either in the laboratory (Haverty et al., 1989; Jones, 1984; Su *et al.*, 1982a; Su and Scheffrahn, 1988, 1991) or the field (Esenther, 1985; Jones, 1989; Su, 1991; Su *et al.*, 1982b). In laboratory tests, Jones (1987) exposed termites to decayed blocks treated with either of two insect growth regulators, but undecayed blocks were not simultaneously tested. Jones (1991) observed that termites fed on decayed, borate- treated wood at 1.2% and 1.4% (wt./wt) in the laboratory, yet in the field, termites scarcely fed on an undecayed, 1.0% borate-treated substrate. Such findings may reflect the termites' preference for fungal-decayed substrates.

In the current study, *R. flavipes'* acceptance of chemically treated decayed wood and sound wood was compared. Two candidate bait toxicants, sulfluramid (GX-071) (Griffin Corp.) and imidacloprid (NTN 33893) (Miles Inc., formerly Mobay Corp.), were evaluated in the laboratory. Sulfluramid has been reported as a slow-acting toxicant by Su and Scheffrahn (1988, 1991). In an extensive series of laboratory and field trials conducted by the manufacturer, imidacloprid has shown promise for subterranean termite control (Zeck, 1992).

MATERIALS AND METHODS

Wood. Lumber from a single sweetgum (*Liquidambar styraciflua* L.) tree was used to prepare all blocks, which were cut to approximately 2.5 by 3.8 by 0.6 cm (mean wt. 3.5 g). One group of blocks was kept sound, and the other group was decayed to approximately 20% weight loss with the Madison 617 isolate of *G. trabeum*, based on the procedures of Amburgey et al. (1981).

Chemical Treatment. Imidacloprid (1-[(6-chloro-3-pyridinyl) methyl]-4,5-dihydro-N-nitro-1-H-imidazol-2-amine) is a nitroguanidine analogue (Altmann, 1990), and sulfluramid (N-ethyl perfluorooctane sulfonamide) is a fluoroaliphatic sulfonamide. Each chemical was evaluated against termites at a concentration of 100 ppm (wt./wt.) in wood.

Decayed and sound wood blocks were oven dried at 105°C for 24 h and then weighed prior to being vacuum impregnated (ASTM, 1986) either with acetone alone (controls) or acetone solutions of technical grade imidacloprid or sulfluramid. Blocks were weighed immediately after treatment to determine chemical uptake; then they were air dried for at least 72 h to allow the solvent to evaporate.

Termites. Large foraging groups, representing three colonies of *R. flavipes*, were collected in logs of southern yellow pine (*Pinus* spp.) from the Desoto National Forest, ca. 20 km north of Gulfport, Mississippi. The infested logs were maintained in the laboratory for 1 to 3 months prior to test initiation. A vacuum aspirator (Jones and Mauldin, 1983) was used to make exact counts of groups of 200 workers and 5 soldiers, which were placed in each experimental unit.

Experimental Units. Six or seven replicates were prepared for each chemical × decay status × colony × time (3 × 2 × 3 × 4) combination for a total of 426 units (excepting missing data when several replicates inadvertently were not recorded at breakdown times). Each unit was scheduled to be dismantled 7, 14, 21, or 28 days after test initiation. Each block was moistened with 13 drops of distilled water and buried in 25 ± 1 g of a mixture of sand:vermiculite:water (1:1:0.42 by volume) (Haverty, 1979) contained in a plastic cylinder (5.0 cm diam by 3.5 cm high). Termites were placed on top of the sand-vermiculite matrix after being randomly assigned to a container, which was coded to mask its treatment status. Experimental units were held in the laboratory at 23 ± 2°C.

The number and condition of termites was noted at each weekly examination, and each block was cleaned with a small brush and air dried. Blocks subsequently were oven dried at 105°C for 24 h and then weighed. The weight loss of each, presumably caused by termite feeding, was calculated after correcting for background loss caused by the experimental conditions.

To determine background weight loss, 142 units containing decayed or sound acetone-treated control blocks (17 to 18 reps/time) and devoid of termites were concurrently established and dismantled using the same protocol as for the units containing termites. Subsequently, an auxiliary experiment was conducted to determine whether background weight loss was influenced by chemical treatment. Using the same protocol, a total of 72 units was established with imidacloprid and sulfluramid-treated blocks, as well as acetone-treated controls.

Data Analyses. Response variables included proportion mortality (transformed) and corrected block weight loss. Termite mortality was based on the proportion of dead workers and presoldiers (which developed from workers) versus live workers (including ataxic and moribund termites). To avoid proportions of 0 and 1, the following formula was used:

$$\text{Prop}_{\text{dead}} = (\text{number dead workers} + \text{presoldiers} + 1)/202.$$

A logit transformation then was used to linearize these proportional data (Hosmer and Lemeshow, 1989).

Two-sample t-tests with a Bonferroni correction were used to compare termite mortality in each treatment with the control. Analysis of variance (ANOVA) then was used to determine the significance of mortality and block weight loss in the controls and in the two chemical treatments. Colony was used as a blocking factor.

RESULTS AND DISCUSSION

Survival. In this no-choice feeding situation, imidacloprid and sulfluramid caused significant reductions in termite survival compared with that of the controls (Fig. 1). In tests with imidacloprid, a few survivors were found at the 7-day examination, and even fewer after 14 and 21 days. No surviving termites were found at the 28-day examination. In tests with sulfluramid, survival was slightly, but significantly, lower than that of the controls at 7 days, and it rapidly declined thereafter. At 28 days, only a few termites survived in several replicates. As revealed in Fig. 1, more dead termites generally occurred in treatments with imidacloprid than sulfluramid at each weekly interval.

No significant differences in termite mortality were evident for any term pertaining to the decay status of treated blocks (Table 1). A similar scenario was observed in the controls, i.e., decay [$F(1, 131) = 0.46$, $P = 0.50$] and time × decay [$F(3, 131) = 1.41$, $P = 0.24$]. Because mortality ensued so

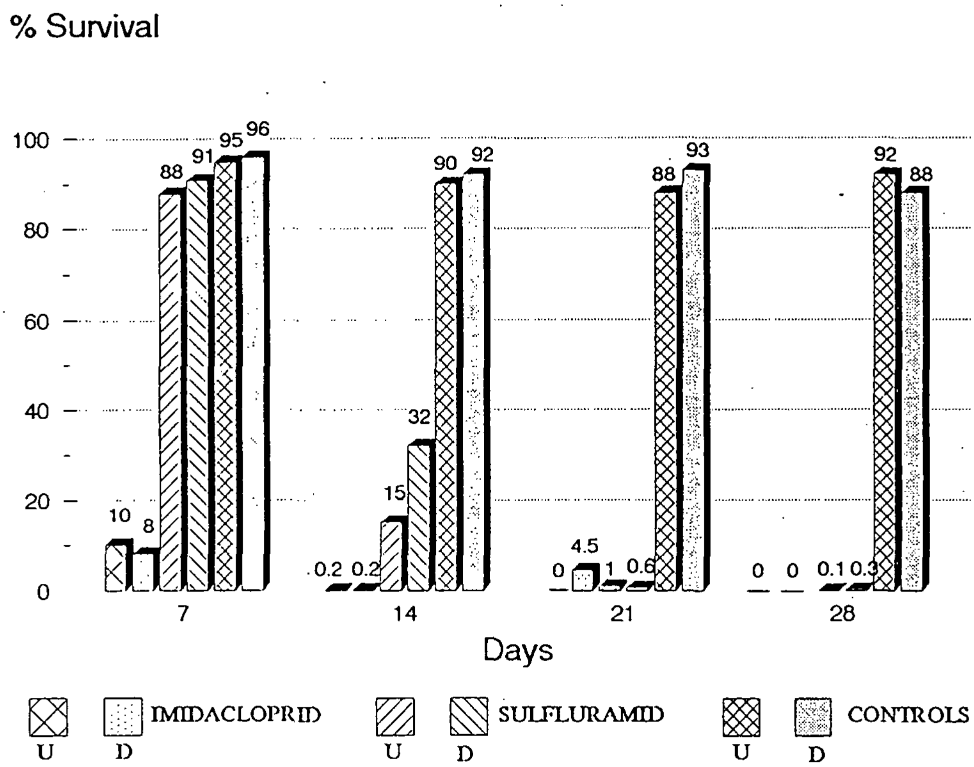

Fig. 1. Average percentage survival of *R. flavipes* after exposure to undecayed (U) and decayed (D) sweetgum blocks treated with imidacloprid, sulfluramid, or acetone (controls) in no-choice feeding tests. At each time, mean survival in either chemical treatment was significantly lower than in the controls ($\alpha = 0.005$).

Table 1. ANOVA for survival of *R. flavipes* after weekly exposure to sound and decayed sweetgum blocks treated with imidacloprid or sulfluramid in no-choice feeding tests ($\alpha = 0.05$)

Source	DF	F value	Pr > F
Chemical	1	231.11	6×10^{-38}
Time	3	188.89	2×10^{-65}
Chem. × time	3	97.08	2×10^{-42}
Decay	1	0.08	0.78
Chem. × decay	1	0.55	0.46
Time × decay	3	0.64	0.59
Chem. × time × decay	3	1.03	0.38
Colony	2	5.79	3×10^{-3}
Error	265		

quickly after termites were exposed to 100 ppm imidacloprid or sulfluramid, any differences attributable to the decay status of the substrate might have been obscured by the lengthy observation period. Nonetheless, the same patterns of statistical significance were observed in an ANOVA restricting attention to survival at days 7 and 14. At 100 ppm of either chemical, the data suggest that the time-consuming process of decaying blocks is not necessary to achieve substantial termite mortality in laboratory no-choice tests.

Background Block Weight Loss. That experimental conditions contributed to the measured weight loss of blocks is evidenced in test setups devoid of termites. Many of the blocks had become infested with mold fungi, which may have contributed to weight loss. A two-way ANOVA indicated significant differences for the main effects of time [$F(3, 134) = 13.74$, $P = 7 \times 10^{-8}$] and decay status [$F(1, 134) = 155.27$, $P = 4 \times 10^{-24}$], as well as for the time-by-decay interaction [$F(3, 134) = 3.10$, $P = 0.03$]. After 7, 14, 21, and 28 days, weight loss of decayed blocks averaged 0.05, 0.10, 0.10, and 0.11 g; and that of undecayed blocks averaged 0.02, 0.03, 0.04, and 0.04 g, respectively. In the auxiliary experiment, no significant differences in weight loss were observed for any term involving the chemical factor (Table 2), which suggested that chemical treatment did not significantly affect background weight loss. In test setups with termites, block weight loss was adjusted by subtracting average background weight loss of the initial set of acetone-treated control blocks.

Decayed control blocks lost 2.5 to 3.3 times more weight than undecayed blocks. Decayed wood may be more susceptible than sound wood to invasion by other decay organisms. For example, research by Holt (1982) demonstrated that when wood blocks were preconditioned by certain wood decaying fungi, particularly the cellulase-less mutants of white rot species, the wood became susceptible to degradation by cellulolytic bacteria and actinomycetes. However, it is more likely that because *G. trabeum* is a Basidiomycete and forms asexual spores on its hyphae (ooidia and chlamydospores), this fungus continued to degrade the blocks even after they were oven dried at a high temperature and impregnated with an acetone solution (M. Gilliam, pers. comm.). A similar scenario may have occurred in tests conducted by Lenz et al. (1991), in which mild heat (40°C for 48 h) was assumed to have sterilized decayed wood blocks. Although not specified by Amburgey et al. (1981), decayed blocks should be autoclaved to kill the fungus (M. Gilliam, pers. comm.) prior to chemical treatment. Gamma irradiation would serve the same purpose (T. Amburgey, pers. comm.).

Table 2 ANOVA for weight loss of sound and decayed sweetgum blocks treated with imidacloprid or sulfluramid or acetone (controls) and placed in test setups devoid of termites for 7, 14, 21, and 28 days ($\alpha = 0.05$)

Source	DF	F value	Pr> F
Chemical	2	0.82	0.45
Time	3	40.20	4×10^{-13}
Chem. × time	6	0.19	0.98
Decay	1	175.36	1×10^{-17}
Chem. × decay	2	2.22	0.12
Time × decay	3	7.91	2×10^{-4}
Chem. × time × decay	6	1.13	0.36
Error	48		

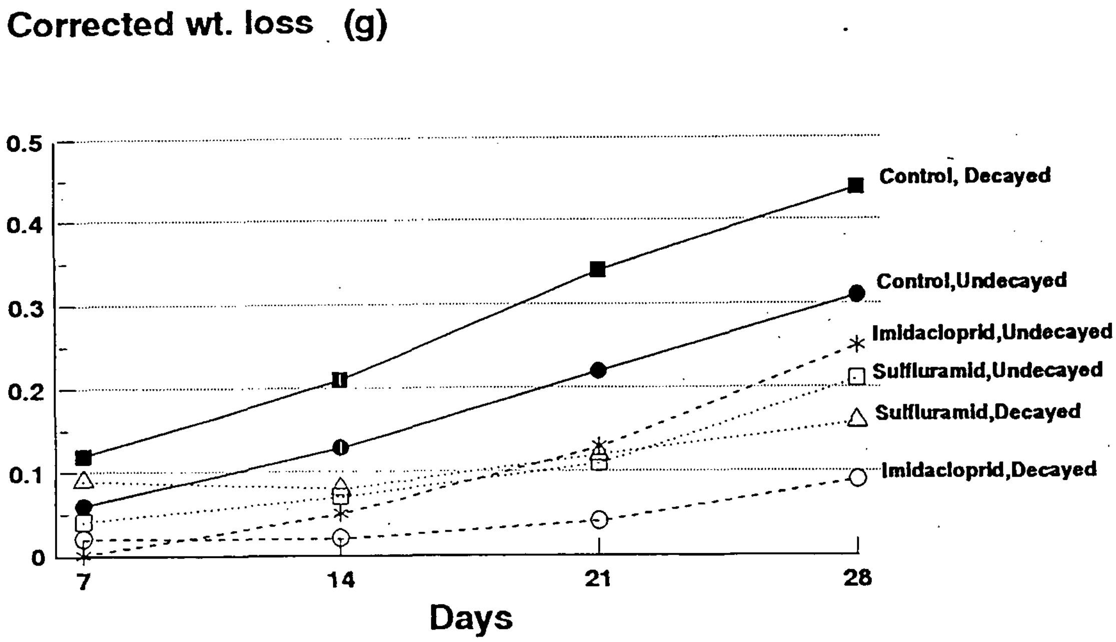

Fig. 2. Average corrected weight loss of sound and decayed sweetgum blocks treated with imidacloprid, sulfluramid, or acetone (controls) and exposed to *R. flavipes* in no-choice feeding tests. The correction factor to determine block weight loss attributable to termites is based on subtraction of average background weight loss of acetone-treated control blocks in test setups devoid of termites.

Alternatively, background weight loss should be calculated to account for any additional wood degradation caused by the decay fungus.

The minor weight losses of undecayed wood blocks also indicate the need to establish such controls without termites. However, the possibility of background weight loss of wood has essentially been ignored by researchers, with the exceptions of Delaplane and La Fage (1990) and Grace et al. (1992). Data in the current study indicate that this variable should be considered when termite consumption of wood is assessed via block weight loss, particularly if associated mould fungi degrade the wood and thus alter its weight. Although most researchers determine termite acceptance of a substrate by comparing the relative weight loss of treatments and controls, reliable comparisons are unlikely since mould fungi occur more readily in units that contain dead termites or that lack healthy termites to feed on and groom the wood (Jones, pers. obs.). The action of mould fungi would lead to an overestimation of termite consumption of the treatment relative to the control, regardless of whether the substrate was sound or previously decayed.

Block Weight Loss Attributable to Termites. The corrected weight loss of control blocks exposed to termites, presumably caused by their feeding, exceeded that of blocks treated with imidacloprid and sulfluramid at each inspection (Fig. 2). This contrast may be primarily attributed to the small number of survivors available to feed on the treated blocks (Fig. 1). Nonetheless, visual inspections of treated blocks generally revealed termite excavations into the wood.In the controls, decayed blocks lost more weight than sound blocks at each time interval (Fig. 2). Such observations are consistent with data published by other researchers regarding favourable termite responses to wood decayed by *G. trabeum*. An ANOVA of corrected weight loss in the controls indicated significant effects for time [$F(3, 131) = 380.77$, $P = 2 \times 10^{-64}$], decay status [$F(1, 131) = 241.81$, $P = 2 \times 10^{-31}$], and the time-by-decay interaction [$F(3, 131) = 7.72$, $P = 9 \times 10^{-5}$]. With time, termites exposed to control blocks consumed more wood, particularly if it was decayed.

The chemical treatments resulted in dramatically decreasing survival as time progressed (Fig. 1). A concomitant decrease in weight loss was expected but was not observed in the two chemical treatments (Fig. 2). Even with dramatic reductions in termite survival, corrected weight loss of the treated blocks increased at each weekly interval. This finding suggests that weight loss may not accurately reflect termite consumption of the treated blocks. However, weight loss of treated blocks at the earliest examinations may be the most reflective of termite consumption, since survivors were

Table 3. ANOVA for corrected weight loss of sound and decayed sweetgum blocks treated with imidacloprid or sulfluramid and exposed for 7 and 14 days to *R flavipes* in no-choice feeding tests ($\alpha = 0.05$)

Source	DF	F value	Pr>F
Chemical	1	130.61	2×10^{-21}
Time	1	21.22	9×10^{-6}
Chem. × time	1	6.59	0.01
Decay	1	4.02	0.05
Chem. × decay	1	15.79	1×10^{-4}
Time × decay	1	24.58	2×10^{-6}
Chem. × time × decay	1	0.28	0.60
Colony	2	1.48	0.23
	Error 134		

Table 4 Least squares means for corrected weight loss (g) of treated sweetgum blocks exposed to *R. flavipes* in no-choice feeding tests

		7 days	*14 days*
DECAYED	Imidacloprid	0.017	0.023
	Sulfluramid	0.086	0.076
	Total	0.103	0.099
UNDECAYED	Imidacloprid	0.003	0.053
	Sulfluramid	0.045	0.070
	Total	0.048	0.123

present. Therefore, an ANOVA was conducted restricting attention to corrected block weight loss at days 7 and 14 (Table 3).

As shown in Table 3, three of the terms involving decay status were statistically significant, with two of the interaction terms highly so. To reveal the relationship between decay status and corrected block weight loss, the least squares means for chemical-by-time-by-decay status are reported in Table 4. The pattern of weight loss of decayed wood reveals that similar losses occurred at 7 and 14 days. For sound wood, greater losses occurred after 14 days than after 7 days. This difference can be explained if termites readily consume decayed wood and mortality quickly ensues, and thus, over time, fewer termites are available to eat the decayed wood as compared with the sound wood. Another data pattern is that decayed wood exhibited greater weight loss than sound wood at day 7, but at day 14, sound wood exhibited slightly greater weight loss than decayed wood. This finding also is consistent with the observation that more termites are alive and feeding at 7 days, and they prefer decayed wood. Thus, both patterns suggest that termites prefer decayed to sound treated wood.

Although relatively greater numbers of surviving termites were present at days 7 and 14, corrected block weight loss continued to increase at days 21 and 28 (Fig. 2). An explanation for this apparent paradox is that large numbers of dead, decaying termites were observed adjacent to the treated blocks at many weekly examinations, and the associated mould fungi possibly contributed to wood degradation and weight loss. This phenomenon would not have been accounted for by correcting for background weight loss in the controls without termites. Preliminary findings suggest that dead, decaying termites contribute to block weight loss, and more extensive studies are under way.

Additional laboratory and field testing of imidacloprid and sulfluramid as bait toxicants is warranted. Data in the current study indicate that shorter time intervals or lower concentrations are needed to further assess termite mortality and wood consumption in the laboratory. Background weight loss of blocks should be considered in all experiments designed to measure wood consumption by termites. Although decayed wood slightly affected termite response to imidacloprid and sulfluramid at 100 ppm, termites may respond more or less favorably when exposed to other chemicals or concentrations. Dramatic differences in mortality and wood consumption would be necessary to justify the time-consuming and labour-intensive process of decaying wood blocks.

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