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Laboratory evaluation of the novel soil insecticide silafluofen against *Coptotermes formosanus* Shiraki (Isopt., Rhinotermitidae)

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Abstract

Effects of the new insecticide silafluofen (HOE 084498) on tunneling and survival of Formosan subterranean termites, *Coptotermes formosanus* Shiraki, were determined in both forced-exposure and indirect-exposure laboratory bioassays. In the indirect-exposure assays, termites were given the option of tunneling through sand treated with an emulsifiable formulation of silafluofen to reach a supplemental food source. Although *C. formosanus* tunneling was reduced in sand containing 10 ppm silafluofen, this concentration did not cause significant mortality in the forced-exposure assay. Forced exposure to 100 ppm silafluofen killed all termites within 24 h. In tunneling assays, 500 ppm silafluofen was required to prevent complete termite penetration through the 7.5 cm tunneling arena, and ≥ 1000 ppm to limit tunneling distances to 1 cm or less. In topical application assays, a 5 % dust formulation of silafluofen proved extremely toxic to *C. formosanus* workers, and may be useful for treatment of termite galleries within wood.

1 Introduction

Subterranean termites (Isopt., Rhinotermitidae) are worldwide pests of wood products, and of living trees and crops. The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is a particularly severe problem in tropical and subtropical regions. This termite is readily transported by commerce and has spread throughout the world, including Hawaii and the southern continental United States (SU and TAMASHIRO 1987). Both laboratory (BEAL and SMITH 1971; JONES 1988, 1990; GRACE 1991) and field observations (MAULDIN et al. 1987) have demonstrated that *C. formosanus* is more difficult to control with chemical soil treatments than a number of other subterranean pest species. Thus, control methods demonstrated to be efficacious against *C. formosanus* may be transferable to other termite species as well, although methods effective against *Reticulitermes* spp. or *Heterotermes* spp. do not necessarily deter *C. formosanus.*

This paper reports the results of a series of laboratory assays to determine the efficacy of the novel silicon-containing insecticide silafluofen (HOE 084498), (4-ethoxyphenyl)(3-(4-fluoro-3-phenoxy-phenyl)-propyl)-silane, as a soil insecticide for the control of the Formosan subterranean termite. We evaluated the contact toxicity of an emulsifiable formulation to *C. formosanus* workers in a forced-exposure (no-choice) assay on chemically treated sand, and measured its repellency and toxicity to *C. formosanus* in an indirect-exposure tunneling assay where termite workers are presented with the option of tunneling through treated sand to reach an alternate food source (GRACE 1990). We also evaluated the contact toxicity of a 5 % dust formulation of silafluofen to *C. formosanus*. Insecticidal dusts may prove useful for contaminating foraging termite workers in bait delivery systems, and for injection into termite galleries in wood or soil (GRACE and ABDALLAY 1990; GRACE et al. 1990).

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2 Materials and methods

2.1 Materials

Two formulations of silafluofen (HOE 084498, Hoechst Aktiengesellschaft, Germany) were evaluated: HOE 084498 EW20, a 200 g/l emulsifiable formulation; and HOE 084498 Dust-5, a 5% silafluofen dust formulation. Silafluofen is a new broad-spectrum insecticide with relatively low mammalian toxicity (rat oral and dermal $LD_{50} > 5000$) and some demonstrated toxicity to wood-boring beetles (Rustenburg and Klaver 1991). The emulsifiable formulation was diluted in distilled water as necessary to achieve the desired concentration of silafluofen in the sand (weight of silafluofen per weight of dry sand, expressed as ppm). For each concentration, 100 g dry sand was added to a beaker containing 20 ml pesticide solution, thoroughly mixed, and left to dry in a fume hood.

Formosan subterranean termites, *Coptotermes formosanus* Shiraki, were collected as needed from an active field colony on the Manoa campus of the University of Hawaii, using the trapping technique described by Tamashiro et al. (1973). Termites were used in bioassays within 8 h of collection. Only workers, undifferentiated individuals older than the third instar (as determined by size), were used in these assays. In tunneling and toxicity assays, the standard tunneling substrate was washed crushed coral sand, sifted to pass a U.S. 20-mesh screen, with pH = 9.6 as determined by the method of Chapman and Pratt (1961). During the assays, termites were maintained in an unlighted temperature cabinet (29 °C, ca 80 % RH).

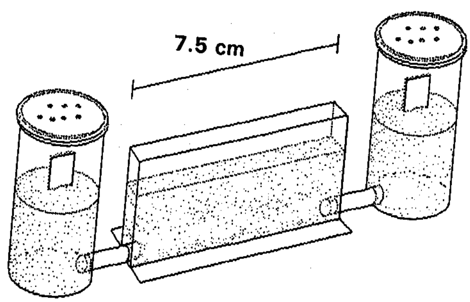
2.2 Contact toxicity

In a forced-exposure (no-choice) assay, a layer (ca 2.5 ml) of dry sand, treated as described above with an aqueous dilution of silafluofen, was placed in a 10 × 35 mm plastic Petri dish (Falcon No. 1008). One ml distilled water was added to the sand, and 30 *C. formosanus* workers were placed in the dish. Termite tunneling activity and mortality were recorded after 1 day and 7 days. Three replicates were performed with each concentration of 0, 1, 10, 100, or 1000 ppm silafluofen.

2.3 Tunneling assays

Indirect-exposure tunneling assays were performed with *C. formosanus* workers as described by Grace (1990, 1991). This assay mimics indirect exposure to pesticide-treated soil in a field situation, in that termites are presented with the option of tunneling through treated sand to reach a second food source. The assay apparatus (see figure) has three compartments: 1. a plastic vial containing untreated sand, termites and cellulosic food, 2. a glass "sandwich", or tunneling arena, containing the treated sand, and 3. a second vial containing untreated sand and an additional food source. These are connected serially by 1.5 cm lengths of Tygon tubing. The sandwich-like tunneling arena consists of two glass microscope slides (2.5 × 7.5 cm) spaced 3–4 mm apart and secured in a horizontal upright position on one long edge by silicone rubber sealant (General Purpose Clear Sealant, Dow Corning Corp., Midland, Michigan) to a third flat glass slide as a base. The ends of the tunneling arena are sealed with plastic spacers and silicone caulking, with a 1.5 cm long Tygon tube at the base of each end of the sandwich leading into the base of one of the two 55 ml polystyrene vials (60 × 35 mm diameter). Each of these vials contains ca 15 g untreated sand, 3 ml water, and a 1.5 × 2.5 cm length of wooden tongue depressor (Puritan No. 25-705, Hardwood Products Co., Guilford, Maine) as food.

The treated sand (ca 7 g) was poured into the tunneling arena, and 1.5 ml distilled water added by pipette along the open top edge. The addition of water visibly moistened the sand to the base of the tunneling arena. The top edge of each tunneling arena was sealed with plaster of paris (to retain moisture), and 50 *C. formosanus* workers placed in one of the adjacent vials. The vials were capped (a



Bioassay apparatus for measuring tunneling by termite workers heated insect pin was used to pierce air holes in the caps), and the three-chamber apparatus placed in the incubator. At 10 days, total tunneling distance in the arena and termite mortality were recorded and subjected to analysis of variance with a one-way layout, and means significantly different at the 0.05 level were separated by the Ryan-Einot-Gabriel-Welsch multiple F test (SAS Institute 1987). Tunneling distances could exceed the 7.5 cm length of the arena due to sinuous tunnels or multiple tunnels within a single sandwich. Percentage mortality data was transformed by the arcsine of the square root prior to analysis.

The first tunneling bioassay evaluated high concentrations of silafluofen: 1000, 2000, 3000, 4000, 5000, and 7500 ppm by weight in the sand. Six replicates, each with 50 *C. formosanus* workers, were included of each concentration and of the untreated controls.

As a result of the efficacy demonstrated in the first tunneling bioassay, a second assay was performed with lower concentrations of silafluofen: 0, 1, 10, 100, and 1000 ppm. Three replicates (50 termite workers per replicate) were included of each concentration.

A third final tunneling bioassay was then performed with the target silafluofen concentrations indicated by the results of the first two assays: 0, 500, 1000, 1500, and 2000 ppm in the sand. Jones (1990) has demonstrated that a large group of Formosan subterranean termites will tunnel further into pesticide-treated soil than a small group, an important finding since *C. formosanus* colonies are characterized by high population densities. In recognition of this, we increased the density of termite workers in each replicate 5-fold from 50 to 250 individuals in this third tunneling assay. Termite tunneling and mortality in three replicates of each silafluofen concentration, each containing 250 *C. formosanus* workers, were evaluated.

2.4 Dust toxicity

Toxicity of the 5 % silafluofen dust formulation to *C. formosanus* was determined using methods similar to those previously described with borate dusts (Grace and Abdallay 1990; Grace et al. 1990; Grace 1991). *Coptotermes formosanus* workers were coated with HOE 084498 Dust-5 by placing a group of 20 workers in a small (4.5 cm diameter) glass Petri dish containing a thin layer of powder (10 mg), gently shaking the dish for 10 sec, then pouring the termites out onto a small weighing paper placed in the center of a larger 9 cm Whatman No. 1 filter paper in a glass Petri dish. After the termites walked off the weighing paper, it was removed, and the dish placed in a plastic box lined with damp paper toweling to maintain humidity. Five groups of 20 dust-treated termite workers were evaluated, along with 5 control groups handled in a similar fashion. Condition of the termites was recorded 1, 2, 3, 4, and 22 h after treatment.

3 Results and discussion

3.1 Contact toxicity

All *C. formosanus* workers placed in Petri dishes on sand containing 100 or 1000 ppm silafluofen died within 24 hours (table 1). No significant mortality was observed from contact with sand containing 1 or 10 ppm silafluofen during the seven day assay period. However, these low concentrations were noted to be repellent to the insects, with a slight decrease in tunneling activity in sand containing 1 ppm, and greatly decreased tunneling in

Table 1. **Contact toxity of sand treated with silafluofen to *C. formosanus* workers in a forced-exposure assay**[1]

Concentration ppm	Day 1 (24 h) Mortality %	Day 1 (24 h) Tunneling activity	Day 7 Mortality %	Day 7 Tunneling activity
1000	100 ± 0a	None	–	–
100	100 ± 0a	None	–	–
10	0 ± 0b	Minor – at edge of dish	4.44 ± 2.22a	Minor – at edge of dish
1	0 ± 0b	Minor – throughout dish	5.56 ± 2.94a	Active – throughout dish
0	0 ± 0b	Active – throughout dish	4.44 ± 1.11a	Very active – throughout dish

[1] Each mean (±SEM) represents 3 groups of 30 workers placed on treated sand. Means within each column followed by the same letter are not significantly different at the 0.05 level.

the 10 ppm treatment (table 1). Repellency at sublethal doses is also observed with pyrethroid termiticides (JONES 1988; SMITH and RUST 1990; SU and SCHEFFRAHN 1990) and is a desirable characteristic in a soil pesticide for use as a chemical barrier to foraging termites. The toxic threshold of silafluofen to *C. formosanus* is between 10 ppm and 100 ppm, comparable to the values reported for *Reticulitermes hesperus* Banks by SMITH and RUST (1990) for the soil termiticides fenvalerate (Tribute 2EC, Velsicol Chemical Corp.) and isofenphos (Pryfon, Mobay Corp.)

3.2 Tunneling assays

In all three sets of tunneling assays (table 2–4), termites failed to penetrate to any significant degree sand containing ≥ 1000 ppm silafluofen. Trends toward higher termite mortality and less tunneling were observed at concentrations exceeding 1000 ppm, but mortality was variable and the different tunneling distances did not differ statistically from the 1000 ppm level. Concentrations of 1, 10, or 100 ppm silafluofen did not prevent penetration of the treated sand, although the termites subsequently tunneled less in the treated sand than in the untreated controls (table 3). Although tunneling was reduced at 500 ppm, termites still tunneled over 4 cm into the treated sand, with subsequent contact toxicity (table 4).

We hypothesize that after establishing a single tunnel through treated sand, *C. formosanus* minimized contact with the pesticide by restricting movements to the single tunnel and not excavating further, and possibly by lining the tunnel with carton material or untreated sand brought from outside the tunneling arena. In contrast, in the untreated

Table 2. **Tunneling and mortality (mean ± SE) of *C. formosanus* workers after 10 days exposure in a tunneling arena assay to sand containing 1000 ppm or more silafluofen[1]**

Concentration ppm	Mortality %	Tunneling cm	Replicates penetrated
7500	60.67 ± 7.37a	0.00 ± 0.00a	0
5000	55.33 ± 6.82ab	0.07 ± 0.03a	0
4000	23.00 ± 5.16c	0.00 ± 0.00a	0
3000	42.67 ± 7.33abc	0.15 ± 0.06a	0
2000	38.00 ± 4.53abc	0.10 ± 0.08a	0
1000	32.33 ± 2.39bc	0.17 ± 0.08a	0
0	6.67 ± 2.46d	6.70 ± 0.12b	6

[1] Each mean (± SEM) represents 6 replicates of 50 workers per treatment. Means within each column followed by the same letter are not significantly different at the 0.05 level.

Table 3. **Tunneling and mortality of *C. formosanus* workers after 10 days exposure in a tunneling arena assay to sand containing 1000 ppm or less silafluofen[1]**

Concentration ppm	Mortality %	Tunneling cm	Replicates penetrated
1000	34.67 ± 6.77a	0.87 ± 0.19a	0
100	14.67 ± 1.76b	6.00 ± 0.45b	1
10	14.67 ± 3.71b	7.53 ± 0.32b	3
1	10.67 ± 0.67b	6.93 ± 0.03b	3
0	10.67 ± 2.40b	15.90 ± 0.96c	3

[1] Each mean (± SEM) represents 3 replicates of 50 workers. Tunnel distances may exceed length of arena (7.5 cm) due to multiple or sinuous tunnels. Means within each column followed by the same letter are not significantly different at the 0.05 level.

Table 4. **Effect of a high density of *C. formosanus* workers in a tunneling arena assay on tunneling and mortality after 10 days exposure to sand treated with silafluofen**[1]

Concentration ppm	Mortality %	Tunneling cm	Replicates penetrated
2000	53.47 ± 3.28ab	0.07 ± 0.03a	0
1500	32.67 ± 9.73b	0.20 ± 0.10a	0
1000	47.07 ± 5.73ab	1.07 ± 0.23a	0
500	75.20 ± 14.68a	4.63 ± 0.93a	0
0	4.40 ± 0.00c	14.50 ± 3.39b	3

[1] Each mean (± SEM) represents 3 replicates of 250 workers. Tunnel distances may exceed length of arena (7.5 cm) due to multiple or sinuous tunnels. Means within each column followed by the same letter are not significantly different at the 0.05 level.

control assays, termite workers continued to establish additional tunnels in the tunneling arena, continually enlarged these tunnel cavities, and gradually removed most of the sand from the tunneling arena.

These laboratory studies indicate that 1000 ppm silafluofen (by weight in the soil) is likely the minimum concentration that could be expected to provide protection from *C. formosanus* penetration. Tunneling, however, was greatly reduced at 500 ppm (table 4), and 100 ppm was toxic within 24 h when termites were confined to the treated sand (table 1). It is not unusual for insecticide concentrations greater than those necessary to control *Reticulitermes* subterranean termites to be required for *C. formosanus* (Su and Scheffrahn 1990), and lesser concentrations of silafluofen may thus prove effective with other termite genera. To have a reasonable margin for error to allow for non-homogenous substrates, inconsistent application techniques, and compound degradation, our studies suggest that the target concentration for field applications should exceed 1000 ppm by weight in the soil.

3.3 Dust toxicity

All *C. formosanus* workers coated with HOE 84498 Dust-5 were immobilized (dead or moribund) within 2 h and dead within 1 day. In contrast, no mortality had occurred in any of the 5 control replicates up to 22 h after initiation of the test. The pesticide treated termite workers were immediately affected, as with the "quick knockdown" characteristic of pyrethroid applications, and immobile (moribund) within 2 hours after dust application. The rapid toxicity of the 5 % dust formulation of silafluofen indicates that a much greater dilution would be necessary for termite baiting applications, where the goal is a slow-acting toxicant that will be transferred from foraging workers to the other colony members (Su et al. 1982). As a supplemental treatment to eliminate termite feeding in a specific area, silafluofen dust could be useful for injection into termite galleries in wood.

3.4 Conclusions

In the forced-exposure assays, where *C. formosanus* workers were placed directly on silafluofen-treated sand, exposure to 100 ppm caused 100 % mortality within 24 h, and the toxic threshold is between 10 ppm and 100 ppm. Although no significant mortality occurred on sand containing 10 ppm silafluofen, termites tunneled only minimally, indicating some repellency at this concentration. In the tunneling assays, all concentrations of silafluofen reduced *C. formosanus* tunneling in comparison to controls, but concentrations greater than 100 ppm were necessary to prevent penetration of the 7.5 cm tunneling arena. Concentrations ≥ 1000 ppm were required to reduce termite tunneling in the treated sand to 1 cm or less, and such concentrations would thus be appropriate for field tests of the efficacy of silafluofen against Formosan subterranean termites.

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Zusammenfassung

Laborversuche über die Wirkung des neuartigen Bodeninsektizids Silafluofen auf die Formosa-Termite Coptotermes formosanus Shir. (Isopt., Rhinotermitidae)

Bei Versuchen mit partiell (streckenweise) mit flüssigem Silafluofen kontaminiertem Sandboden bewirkten alle Konzentrationen des Wirkstoffs eine Reduktion der Wühltätigkeit der Termite, doch waren 100 ppm und mehr notwendig, um eine 7,5 cm dicke Erdschicht vor der Durchtunnelung zu bewahren. Erst Konzentrationen von 1000 ppm und mehr schützten eine 1 cm breite Erdschicht vor der Durchtunnelung, so daß diese Konzentration notwendig erscheint, um im Freiland die erdwühlende Termite von dem Angriff auf ein Nahrungssubstrat abzuhalten. Bei topikaler Applikation führten 5 % Silafluofen-Staub zur vollständigen Vernichtung der Termite, so daß diese Form der Applikation sich zur Bekämpfung des Schädlings in Holztunnels eignen dürfte.

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