Semiochemicals Extracted from a Dolichoderine Ant Affects the Feeding and Tunneling Behavior of the Formosan Subterranean Termite (Isoptera: Rhinotermitidae)

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J. Econ. Entomol. 87(3): 705–708 (1994)

ABSTRACT Bioassays were performed to test the responses of the Formosan subteranean termite, *Coptotermes formosanus* Shiraki, to dichloromethane extracts of whole *Iridomyrmex glaber* (Mayr) workers. Termite workers were strongly repelled by filter papers treated with *I. glaber* extracts. In a choice test, termite workers fed significantly less on filter papers treated with *I. glaber* extract compared with solvent-treated controls within a 24-h period. Bioassays also were performed to evaluate the effect of different concentrations of ant extract on termite tunneling behavior. The rate at which termites penetrated the treated sand was concentration dependent. In 10 d, termites failed to penetrate any of the sand barriers treated with either 250 or 500 ant equivalents per gram of sand. Our results suggest that ant semiochemicals are a potential source of natural products that could prove useful for termite control.

KEY WORDS *Coptotermes formosanus*, *Iridomyrmex glaber*, semiochemicals

THE FORMOSAN SUBTERRANEAN TERMITE, *Coptotermes formosanus* Shiraki, is the most destructive insect pest in Hawaii. Estimated costs exceed $60 million each year to control infestations and repair damage caused by these termites (Yates & Tamashiro 1990). Efforts to control infestations of the Formosan subterranean termite have relied primarily on insecticide treatments to soil. However, cyclodiene insecticides, which were used widely to control termite infestations in Hawaii in the past, are no longer available, and the alternative insecticides that have replaced the cyclodienes are less persistent (Tamashiro et al. 1990, Grace et al. 1993). Hence, current research is focusing on the efficacy of novel insecticides and development of new methods for termite control.

Ants are the greatest insect enemies of termites. Many species of ants prey on termites opportunistically, and several ant genera are specialized termite predators (Deligne et al. 1981, Holldobler & Wilson 1990). We have observed several species of ants in Hawaii that will prey on termites and nest in termite galleries in wood.

Ants often use semiochemicals as a defense against other insects. The anal-gland secretions of the subfamily Dolichoderinae contain terpenoids that are known to have insecticidal activity (Cavill & Houghton 1974, Cavill et al. 1976, 1982). Our study examined the potential for using semiochemicals produced by the dolichoderine ant *Iridomyrmex glaber* (Mayr) as repellents against termites.

Materials and Methods

Termite and Ant Collections. Formosan subterranean termites were collected on the Manoa campus of the University of Hawaii using a trapping technique described by Tamashiro et al. (1973) in which foraging termites were collected in boxes constructed of Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) lumber. A wooden stake was placed in the ground and covered by a wooden box. The box was protected by a 22.5 liter cylindrical metal can, with the bottom removed, that was placed over each box and covered with a metal sheet. A single termite colony was used throughout this study because earlier tests (unpublished data) established that different *C. formosanus* colonies in Hawaii responded similarly to *I. glaber* extract.

Because *I. glaber* ants frequently invade our termite collection traps and nest in termite galleries, ant colonies were collected from termite traps and maintained in the laboratory at ambient conditions (25°C) in uncovered 30-by-16-cm plastic boxes. Ant colonies collected in the field were composed of hundreds of workers, several queens, and brood. The sides of the boxes were coated with Fluon (Northern Products, Woonsocket, RI) to prevent ants from escaping. Ants were provided with a constant supply of water from a water-filled 15-dram plastic vial that contained small holes in the sides of the container and was positioned upside down in the ant box so that ants could collect water droplets when needed. Each box also contained a cap of a 17-by-100-mm plastic Falcon test tube filled with honey. Freshly killed termites were added to boxes for protein as necessary. Termites were killed by heating them in a microwave for 20 s.

Feeding Deterrence Tests. Extracts of *I. glaber* workers were made by killing ants on dry ice and soaking 500 ants in 1 ml of dichloromethane for 15 min. In each experiment, 10 μl of ant extract was applied by micropipette to a 1-cm-diameter filter paper disk (Whatman no. 1) (one ant equivalent per mg of paper). After the observation period, a 5.5-cm-diameter filter paper disk (Whatman no. 1) was moistened with distilled water and taped to the inside cover of the glass dish to provide moisture for the termites. Dishes were placed in an unlit temperature cabinet (28°C, 80% RH) overnight, removed after 24 h, oven-dried (90°C for 2 h), and weighed to determine the amount eaten.

In the first experiment, termites were presented with a choice of extract-treated or solvent-treated disks. The two disks were weighed and then placed on opposite sides of a 4.5-cm-diameter glass petri dish. There were 20 replicates (100 termite workers per replicate) for each test. The number of workers in contact with each filter paper disk was counted every half hour over a 3-h period (n = 6 counts), and the total number of termites in contact with each disk was summed over the 3-h period. These totals were compared using a paired comparisons t-test (SAS Institute 1987). Each experiment was performed with the same group of termites for 3 d to determine if the termites would habituate to the ant extract. Each day, new extract-treated and solvent-treated filter paper disks were placed in each petri dish, and termite behavior was monitored every half hour for a 3-h period. This experiment was repeated three times. The weight losses of treated and control disks were analyzed using a paired comparisons t-test (SAS Institute 1987).

In the second experiment, termites were presented with either a single extract-treated or a solvent-treated paper disk. There were 10 replicates (100 termite workers per replicate) for both extract-treated disks and solvent-treated controls. The number of workers in contact with each filter paper disk was counted every 10 min for 1 h (n = 6 counts), and the total number of termites in contact with each disk was summed over the 1-h period. The termites then were incubated as described. Weight losses of extract-treated and solvent-treated disks were compared using a t-test (SAS Institute 1987).

In the third experiment, termites also were presented with either a single extract-treated or a solvent-treated paper disk. However, *I. glaber* workers were soaked in dichloromethane for 24 h to improve the efficacy of the extraction. Filter paper disks (Whatman no. 1) 1 cm in diameter were treated with 10 μl of *I. glaber* extract at concentrations of 10 and 0.5 ant equivalents per mg of paper or with the solvent alone, then moistened with 10 μl of distilled water and placed in the bottom of a plastic 17-by-100-mm test tube (Falcon 2057). Thirty *C. formosanus* workers were placed in each test tube. The tubes were capped, and the termites then were incubated as described. There were 10 replicates for both extract-treated and solvent-treated disks. Weight losses of extract-treated and solvent-treated disks were compared using a t-test (SAS Institute 1987). Termite mortality also was recorded after 24 h. Percentage mortality data were transformed by the arcsine of the square root and compared using a t-test (SAS Institute 1987).

Tunneling Bioassay. An indirect exposure tunneling bioassay (Grace 1991, Grace et al. 1992) was used to evaluate the effect of sand treated with different concentrations of ant extract on termite tunneling behavior. Ants were soaked in dichloromethane for 24 h. Two grams of silica sand (Silica 5151 [fine granular silcon dioxide], Fisher Scientific, Springfield, NJ) were treated with 500 μl of extract (applied by pipette) at concentrations of 62.5, 125, 250, or 500 ant equivalents per gram of sand.

The assay apparatus had three compartments: (1) a plastic 15-dram vial containing 17 g of untreated sand, 3 ml water, and a 1.5-by-2.5-cm length of wooden tongue depressor (Puritan No. 25-705, Hardwood Products, Guilford, ME) as food; (2) a glass *sandwich*, or tunneling arena, containing the treated sand; and (3) a second plastic 15-dram vial also containing 17 g of untreated sand, 3 ml water, and a 1.5-by-2.5-cm length of wooden tongue depressor as food. The sandwichlike tunneling arena consisted of two glass microscope slides (2.5 by 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., Midland, MI) to a third flat glass slide as a base. The ends of the tunneling arena were sealed with plastic spacers and silicone caulking, with a Tygon tube 1.5-cm long at the base of each end of the sandwich leading into the base of one of the two 55-ml polystyrene vials (60 by 35 mm diameter). Each compartment was connected serially by 1.5-cm lengths of 5-mm-diameter Tygon tubing.

The extract-treated sand (2 g) was allowed to dry for approximately 1 h to evaporate the solvent, then poured into the tunneling arena. The top edge of each tunneling arena was sealed with plaster of paris, and 100 termites (90 workers, 10 soldiers to approximate natural caste proportions) were placed in one of the adjacent vials.

Table 1. Mean (±SD) numbers of *C. formosanus* workers in contact with filter papers treated with a dichloromethane extract of *I. glaber* (one ant equivalent per mg) or with solvent-treated control papers in feeding tests

Test[a]	Days of exposure	No. of termites in contact with paper disk[b]		$P > t^c$
		Treatment	Control	
Choice 1	0	52.2 ± 19.1	101.7 ± 15.9	0.0004
	1	20.5 ± 7.5	124.2 ± 9.8	0.0001
	2	2.3 ± 2.1	117.8 ± 6.0	0.0001
Choice 2	0	14.7 ± 5.6	47.0 ± 12.3	0.0004
	1	9.8 ± 3.1	171.5 ± 10.8	0.0001
	2	4.2 ± 4.7	162.8 ± 10.3	0.0001
Choice 3	0	17.8 ± 6.1	73.3 ± 16.4	0.0007
	1	24.0 ± 4.5	80.2 ± 10.1	0.0004
	2	12.0 ± 6.4	97.2 ± 20.5	0.0002
No-choice	0	1.1 ± 1.3	35.2 ± 6.1	0.0001

[a] Choice test (20 replicates of 100 termites per replicate in each test), and the no-choice test (10 replicates of 100 termites). Paper disks were replaced daily in choice test.
[b] Mean (±SD) number of termites in contact with paper disks summed for all replicates at each half-hour interval (n = 6 intervals).
[c] Differences in treatment and control means were significant (paired comparisons t-test for choice tests and unpaired t-test for no-choice test).

There were six replicates of each concentration, except for the concentration of 500 ant equivalents, of which there were three replicates and 10 replicates of the solvent-treated controls. The vials were capped with plastic lids containing small air holes, and the three-chamber apparatus was placed in an unlit temperature cabinet (28°C, 80% RH). The cumulative distance penetrated by termites through the sand barrier in the arena was measured daily for 10 d. Termite mortality also was recorded after 10 d.

Total tunneling distances in the arenas were compared for 1, 5, and 10 d using an analysis of variance (ANOVA). Means were analyzed using Tukey's studentized range test (SAS Institute 1987). Percentage mortality data after 10 d were transformed by the arcsine of the square root, analyzed by ANOVA, and compared by Tukey's studentized range test.

Results and Discussion

Feeding Deterrence Tests. In the choice test, termite workers avoided contact with disks treated with the *I. glaber* extract ($P < 0.001$). The number of termites in contact with the solvent-treated control disk significantly exceeded the number on the extract-treated disk (Table 1). Termites stayed primarily on the side of the dish opposite the treated filter paper and only occasionally walked across the treated disk. Within a 24-h period, termite workers fed significantly less on disks treated with *I. glaber* extract than on the solvent-treated control disks (Table 2). Grace (1989) demonstrated that termites can habituate to semiochemical stimuli. However, *C. formosanus* workers exposed each day to freshly treated paper disks did not show any decrease in avoidance behavior over a 3-d period.

Table 2. Mean (±SD) weight losses from paper disks treated for 15 min with a dichloromethane extract of *I. glaber* (one ant equivalent per mg) compared with solvent-treated control papers after 24 h of feeding by termite workers

Test	Wt loss (mg)		$P > t$
	Treatment	Control	
Choice 1	0.93 ± 0.95	4.54 ± 1.65	0.0001*
Choice 2	0.79 ± 0.96	2.10 ± 0.98	0.0002*
No choice	2.68 ± 0.99	2.42 ± 0.78	0.26

* Differences in treatment and control means were significant (paired comparisons t-test for choice tests and unpaired t-test for no-choice test).

In the first no-choice test, termites initially avoided contact with the extract-treated disks. During the first hour after treatment, the number of termites on the solvent-treated control disks significantly exceeded the number on the extract-treated disks (Table 1). However, with only a single food source available, termite consumption of extract-treated and control disks did not differ over the 24-h test period (Table 2).

In the second no-choice test in which ants were extracted for 24 h instead of 15 min and termites were enclosed in test tubes, termite workers fed significantly less on filter paper disks treated with *I. glaber* extract than on the solvent-treated control disks for both concentrations tested (Table 3). The difference in termite feeding behavior in this test may have resulted either from the extraction of more deterrent chemicals or from less volatilization of the extract under the more confined conditions of this test.

Tunneling Bioassay. Termites initially were repelled by contact with extract-treated sand. Termites backed up and turned around upon contacting extract-treated sand, even at the lowest concentration tested (62.5 ant equivalents per gram of sand). In contrast, termites began tunneling through the sand in most of the control arenas within a few hours. Within 24 h, termites completely penetrated through sand treated only with solvent in eight of 10 replicates.

Table 3. Mean (±SD) weight losses from paper disks treated for 24 h with a dichloromethane extract of *I. glaber* compared with solvent-treated control papers after 24 h of feeding by termite workers in a no-choice test[a]

Concn (ant equivalents per mg)	Wt loss (mg)		$P > t^b$
	Treatment	Control	
10	0.11 ± 0.25	1.35 ± 0.95	0.002
0.5	0.47 ± 0.95	1.53 ± 0.45	0.007

[a] No-choice test conducted in plastic test tubes (10 replicates of 30 termites).
[b] Differences in treatment and control means were significant (t-test).

Table 4. Mean distance (±SD) penetrated by *C. formosanus* workers through sand in a tunneling arena 7.5 cm long treated with a dichloromethane extract of *I. glaber*

Concn[a]	Distance penetrated, cm			% mortality[b]
	Day 1	Day 5	Day 10	
Control	6.5 ± 2.4a	7.5 ± 0.0a	7.5 ± 0.0a	11.3 ± 9.0a
62.5	0.2 ± 0.4b	2.7 ± 1.7b	7.4 ± 0.2a	16.5 ± 6.1a
125	0.0 ± 0.0b	1.2 ± 0.4c	6.0 ± 1.7a	17.8 ± 7.7a
250	0.0 ± 0.0b	0.6 ± 0.4c	1.7 ± 1.4b	8.8 ± 7.3a
500	0.0 ± 0.0b	0.3 ± 0.6c	3.0 ± 0.0b	18.0 ± 6.1a

Means within a column followed by the same letter are not significantly different (ANOVA; Tukey's studentized range test; $F = 34.39$, df = 4, $P < 0.0001$ for day 1; $F = 105.09$, df = 4, $P < 0.0001$ for day 5; $F = 41.37$; df = 4, $P < 0.0001$ for day 10; $F = 1.65$, df = 4, $P = 0.19$ for percentage mortality.

[a] Ant equivalents per gram of sand.

[b] Mean (±SD) percentage mortality after 10 d.

The rate at which termites penetrated the treated sand was concentration dependent (Table 4). Termites did not tunnel into sand treated with 250 or 125 ant equivalents per gram for 2 d. At 500 ant equivalents per gram of sand, there was no penetration of sand for the first 4 d, and less than half the arena (3 cm) was penetrated after 10 d (Table 4). After 5 d, the distance tunneled in controls was significantly greater than that tunneled in any concentration of extract-treated sand. However, after 10 d the distance tunneled in controls only exceeded that tunneled in sand containing 250 or 500 ant equivalents per gram. At a concentration of 62.5 ant equivalents, termites completely penetrated the sand barrier in five of the six replicates after 10 days, whereas termites only completely penetrated two of the replicates at the 125 ant-equivalent concentration and none at the 250 and 500 ant-equivalent concentrations. After 10 d, termite mortality was uniformally low and did not differ among treatments (Table 4).

Our results demonstrate that ant semiochemicals are a source of naturally produced chemicals that could potentially be of value as termite repellents in wood treatments or soil applications. Research is continuing to identify the active compounds in *I. glaber* secretions and further explore their potential as a new tool for termite management.

Acknowledgments

We are grateful to R. T. Yamamoto for assistance with termite and ant collections and to N. Reimer for identification of ant species. Funding was provided by USDA Specific Cooperative Agreement 58-6615-9-012. This is Journal Series No. 3862 of the Hawaii Institute of Tropical Agriculture and Human Resources.

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Received for publication 18 May 1993; accepted 15 December 1993.