Toxicity of Monoterpenoids and Other Natural Products to the Formosan Subterranean Termite (Isoptera: Rhinotermitidae)

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ABSTRACT Monoterpenoids are potentially useful for the development of new insecticides because of their low mammalian toxicity. Different monoterpenoids differ significantly in their toxicity to particular insects. We evaluated the toxicity of 6 monoterpenoids, 2 alkaloids, and 1 hydrocarbon that are plant or ant semiochemicals to the Formosan subterranean termite, *Coptotermes formosanus* Shiraki. Bioassays were performed to determine the effects of the semiochemicals on the mortality and tunneling behavior of termites and the fumigant activity of 3 monoterpenoids, citral, geraniol, and eugenol. We also tested the effect of the monoterpenoid alcohol eugenol, as a feeding deterrent. We found significant differences in the termiticidal activity of the different compounds tested. Monoterpenoid alcohols, particularly eugenol, were the most effective as termiticides against *C. formosanus*. Eugenol was also more effective as a fumigant than citral and geraniol. In addition, termites did not tunnel through eugenol and geraniol-treated sand barriers for at least 5 d. However, eugenol was not effective as a feeding deterrent when applied directly to blocks of wood, except at the relatively high concentration of 100,000 ppm.

KEY WORDS *Coptotermes formosanus*, monoterpenoids, alkaloids, insecticides, fumigants

MONOTERPENOIDS ARE 10-carbon compounds that occur widely in plants. These compounds are the principal components of essential oils of many higher plants (Harborne and Tomas-Barberan 1991). Monoterpenoids are primarily lipophilic compounds; they act as toxins, feeding deterrents, and oviposition deterrents to a large variety of insects (Gershenzon and Croteau 1991). These natural compounds are potentially useful for the development of new insecticides because of their low mammalian toxicity. For example, pyrethroids are a group of monoterpene esters found in the leaves and flowers of *Chrysanthemum* spp. that have been modified and developed as highly successful commercial insecticides (Pickett 1991). Certain monoterpenes are recognized as safe for humans by the Food and Drug Administration and are used commercially as artificial flavorings and perfumes (Coats 1994).

Although many monoterpenoids have insecticidal properties, the degree of toxicity of different compounds to one species varies considerably (Rice and Coats 1994), as does the susceptibility of different species to the same compounds. For example, the compound *d*-limonene is acutely toxic to southern pine beetles, *Dendroctonus frontalis* Zimmerman (Coyne and Lott 1976), and the cat flea, *Ctenoccephalides felis* (Bouché) (Hink and Fee 1986), but has low topical toxicity to the German cockroach, *Blattella germanica* (L.), and house fly, *Musca domestica* (L.) (Karr and Coats 1988, 1992). The aldehydes, citral and citronellal, are more effective than alcohols such as geraniol and verbenol against the house fly, but less effective against the southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber (Rice and Coats 1994).

Arthropods also produce monoterpenoids (Blum 1981). Ants secrete monoterpenes that act as repellents to other ant species (Scheffrahn et al. 1984) and as alarm pheromones to other colony members (Hölldobler and Wilson 1990). Because ants are known to use monoterpenes as a chemical defense, ant-derived monoterpenes might be effective against insect species. The dolichoderine ant *Ochetellus glaber* Mayr secretes a monoterpene, dolichodial, that is toxic and repellent to the Formosan subterranean termite in treated sand (Cornelius and Grace 1994, Cornelius et al. 1995).

In the study described here, we evaluated the toxicity of 6 monoterpenoids, citral, citronellal, eugenol, geraniol, limonene, and nerol to *C. formosanus*. Except for eugenol, 5 of these monoterpenoids have been recognized as alarm pheromones in ants (Hölldobler and Wilson 1990). We also tested the effects of *n*-undecane, a hydrocarbon that is an alarm pheromone for many ant species in the subfamily Formicinae (Bradshaw et al. 1975, Löfqvist 1976, Hefetz and Orian 1982) and 2 alkaloids—piperidine and pyrrolidine—that are

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found in the poison gland secretions of ants in the genera *Solenopsis* and *Monomorium* and have been demonstrated to be toxic to termites (Clément et al. 1986, Escoubas and Blum 1990).

Materials and Methods

Termite Collections. Formosan subterranean termites were collected on the Manoa campus of the University of Hawaii by using a trapping technique described by Tamashiro et al. (1973), in which foraging termites were collected in boxes constructed of Douglas-fir, *Pseudotsuga menziesii* (Mirbel) Franco, lumber.

Toxicity of Semiochemicals in Treated Sand. The mortality of termites after 48 h exposure to the following semiochemicals was tested in bioassays with treated sand: citral (0.89 g/ml), citronellal (0.85 g/ml), eugenol (1.07 g/ml), geraniol (0.88 g/ml), (+) limonene (0.84 g/ml), nerol (0.88 g/ml), *n*-undecane (0.74 g/ml), piperidine (0.86 g/ml), and pyrrolidine (0.86 g/ml) (Sigma, St. Louis, MO). Compounds were diluted in acetone to achieve concentrations of 10, 1.0, and 0.2 μl/g of sand, compared with controls (solvent only). Eugenol and geraniol were also tested at concentrations of 0.16, 0.12, 0.08, 0.06, 0.04, and 0.02 μl/g of sand.

Treated silica sand (Silica 5151 [fine granular silicon dioxide], Fisher, Pittsburgh, PA) was allowed to dry at ambient conditions in uncovered containers until the solvent evaporated and 5 g of sand were placed in the bottom of a plastic Falcon test tube (17 by 100 mm). Sand was moistened with 100 μl of distilled water and 30 *C. formosanus* workers were placed in each test tube. Termites were in direct, continuous exposure with treated sand for the duration of the experiment. The tubes were capped loosely so that they were not airtight, and placed in an unlit temperature cabinet (28°C, 80% RH) for 48 h. Each treatment included 10 replicates (30 termites per replicate). Proportional mortality, transformed by the arcsine of the square root, was subjected to analysis of variance (ANOVA) by the general linear models procedure. Means were separated by Ryan–Einot–Gabriel–Welsch multiple *F* test (SAS Institute 1987).

Tunneling Bioassays. An indirect exposure tunneling bioassay was used to evaluate the effect of treated sand on termite tunneling behavior. The assay apparatus consisted of 3 plastic vials (60 by 35 mm diameter) connected in series by 10-cm lengths of Tygon tubing (5 mm diameter) inserted through a hole drilled through the side at the base of each vial. The 1st and 3rd vials contained 5 g of untreated silica sand, moistened with 1 ml of distilled water, and a wooden tongue depressor (1.5 by 2.5 cm long) (Fisher, Pittsburgh, PA) as food. The 2nd vial, in the middle of the 3, contained 10 g of treated sand, moistened with 2 ml of distilled water. For each replicate, 100 *C. formosanus* (90 workers, 10 soldiers) were placed in the 1st vial. Thus, these termites would have to tunnel through the treated sand in the middle vial to reach the 3rd vial and explore the entire apparatus. Each treatment included 10 replicates.

In the 1st experiment, the sand was treated with citral, citronellal, limonene, eugenol, geraniol, nerol, or piperidine, diluted in acetone, at a concentration of 0.5 μl/g of sand. The sand was treated 24 h before the experiment began and left to dry at ambient conditions in uncovered containers to allow the solvent to evaporate. In the 2nd experiment, the sand was treated with eugenol, geraniol, nerol, or piperidine at a concentration of 1 μl/g of sand. The sand was treated 10 d before the bioassay and left in uncovered containers at ambient conditions to estimate the longevity of the repellent effect of chemicals in the sand in the absence of the possible confounding factor of termite habituation. Observations of tunneling behavior were made daily; termite mortality was recorded after 72 h and analyzed as described above.

Based on the results of the first 2 tunneling assays, a 3rd bioassay was done to test the effect of eugenol on termite tunneling behavior during a longer 25-d exposure period. These tests were performed in plastic screwtop containers (8 by 10 cm diameter). Container lids were left on loosely so that the containers were not airtight. Two layers of sand (100 g each) were separated by a corrugated cardboard disk (7 cm diameter). The top layer was treated with eugenol in acetone solution at a concentration of 0.2 and 0.16 μl/g sand or acetone alone; the bottom layer was untreated. Sand was moistened with 40 ml of distilled water. Four hundred termites (360 workers, 40 soldiers) contained in a plastic Falcon petri dish (60 by 15 mm diameter) covered with a layer of parafilm were placed on top of a thin layer of untreated sand (50 g) on the bottom of the container.

The petri dish containing termites was placed on top of the sand with the parafilm layer facing down, covered with the remaining untreated sand, followed by the cardboard disk. The treated sand (100 g) was placed on top of the cardboard disk and the sand was moistened with 40 ml of distilled water before the lid was loosely replaced. Thus, termites were protected from the weight of the sand in the top layer by the petri dish and were free to tunnel out of the petri dish through the parafilm and into the untreated sand in the bottom layer of the container. A block of Douglas-fir, *Pseudotsuga menziezii* (Mirbel) Franco, wood (25 by 25 by 5 mm) was placed on top of the treated sand in the center of the container so that termites would have to tunnel through treated sand to feed on the block. The cardboard disk served as a barrier between the treated and untreated sand and as an additional food source for the termites. Termites had to tunnel up from the bottom of the container through the treated sand to reach the wood block. Five replicates (400 termites per replicate) were included in each treatment. Tunneling behavior was observed every 3 d during the test

Table 1. Mean ± SD percentage of termite mortality after 48 h in 5 g of treated sand in a test tube

Treatment	Concn of sand, μl/g		
	10	1	0.2
Control	3.3 ± 2.3b	0.6 ± 1.4b	4.0 ± 4.3c
Citral	100.0 ± 0.0a	94.6 ± 6.0a	2.0 ± 1.8c
Citronellal	100.0 ± 0.0a	8.0 ± 5.5b	0.6 ± 1.4c
Eugenol	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
Geraniol	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
Limonene	4.6 ± 2.9b	4.0 ± 4.3b	2.0 ± 2.9c
Nerol	100.0 ± 0.0a	100.0 ± 0.0a	12.0 ± 5.0b
Piperidine	2.6 ± 2.7b	3.3 ± 4.7b	3.3 ± 3.3c
Pyrrolidine	100.0 ± 0.0a	4.0 ± 3.6b	4.0 ± 2.7bc
n-Undecane	3.3 ± 3.3b	0.6 ± 1.4b	1.3 ± 2.9c

Five replicates (30 termite workers per replicate) were included for each treatment. Means followed by the same letter within columns are not significantly different ($P > 0.05$; Ryan–Einot–Gabriel–Welsch multiple F test [SAS Institute 1987]).

period and termite mortality was recorded after 25 d. Proportional termite mortality data were analyzed as previously described. Differences in the initial and final oven dry weights of the wood blocks among treatments were subjected to ANOVA by the general linear models procedure. Means were separated by Ryan–Einot–Gabriel–Welsch multiple F test (SAS Institute 1987).

Fumigant Activity. The previous bioassays determined the toxicity of chemicals based on both exposure to fumes and direct contact to liquids in treated sand. The fumigant activity of citral, geraniol, and eugenol in treated sand was determined with a bioassay in which termites had no direct contact with the sand and were exposed only to the volatile compounds in the container. Sand was treated as described above, and 100 g of treated sand, moistened with 20 ml of distilled water, was placed in each plastic container (8 by 10 cm diameter), resulting in a head-space volume of 402 cm^3 above the sand (although the containers were not airtight).

In each container, an uncovered plastic Falcon petri dish (60 by 15 mm diameter) containing 30 termite workers was placed on top of the layer of sand. Because termites were not able to climb out of the dish, they did not have any direct contact with the treated sand and were exposed only to volatiles. Containers were placed in an unlit temperature cabinet (28°C, 80% RH) for 48 h. In preliminary tests, total mortality occurred after 48 h from sand treated with 10 μl/g of sand (10.7–8.8 μg/g of sand) for all 3 compounds. Based on these results, tests were done using sand treated with 1.0, 0.8, 0.5, 0.1 μg/g of sand and a control treated with solvent only. We used 5 replicates (30 termites per replicate) of each treatment. Proportional mortality data after 48 h were analyzed as previously described.

Feeding Deterrence Tests. Tests were performed in loosely capped plastic screwtop containers (8 by 10 cm diameter). We placed 200 termites (180 workers, 20 soldiers) and 100 g of sand moistened with 20 ml of distilled water in each container. Test blocks of Douglas-fir wood (25 by 25 by 5 mm) were soaked in a solution of eugenol in acetone or in acetone alone as a control. Termites were exposed to 1 test block, placed on top of the layer of sand in each container in a no-choice test. Five replicates (200 termites per replicate) were used for each treatment. Wood blocks were soaked in solutions at a concentration of 1,000, 10,000, or 100,000 ppm of eugenol for 3 h, left to dry at ambient conditions for 24 h, and placed in a container with termites for 20 d. Differences in the initial and final oven dry weights of the wood blocks among treatments were subjected to ANOVA by the general linear models procedure. Means were separated by Ryan–Einot–Gabriel–Welsch multiple F test (SAS Institute 1987). Proportional mortality data were analyzed as previously described.

Results

Toxicity of Semiochemicals in Treated Sand. We observed 100% mortality when *C. formosanus* was exposed to citral, citronellal, eugenol, geraniol, nerol, and pyrrolidine at a concentration of 10 μl/g of sand, whereas no difference in mortality occurred among termites exposed to limonene, *n*-undecane, piperidine, or controls at that concentration (Table 1). Citral, eugenol, geraniol, and nerol also caused significantly greater termite mortality at 1 μl/g of sand. Eugenol, geraniol, and nerol caused significantly greater termite mortality at 0.2 μl/g of sand (Table 1). Eugenol and geraniol were significantly more toxic than the other semiochemicals tested. Eugenol caused 100% mortality at concentrations as low as 0.06 μl/g of sand and geraniol as low as 0.12 μl/g (Table 2).

Tunneling Bioassays. Termites did not penetrate through the 2nd vial in any of the replicates treated with citral, eugenol, geraniol, or nerol after

Table 2. Mean ± SD percentage of termite mortality after 48 h in 5 g of treated sand in a test tube

Treatment	Concn of sand, μl/g					
	0.16	0.12	0.08	0.06	0.04	0.02
Control	4.6 ± 2.9b	2.0 ± 2.9b	0.6 ± 1.4c	2.0 ± 2.9b	8.0 ± 7.3b	5.3 ± 3.8a
Eugenol	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	58.6 ± 26.9a	2.6 ± 4.3a
Geraniol	100.0 ± 0.0a	100.0 ± 0.0a	68.0 ± 41.9b	4.6 ± 5.0b	2.6 ± 4.3b	1.3 ± 1.8a

Five replicates (30 termite workers per replicate) were included for each treatment. Means followed by the same letter within columns are not significantly different ($P > 0.05$; Ryan–Einot–Gabriel–Welsch multiple F test [SAS Institute 1987]).

Table 3. Toxicity and repellency of semiochemicals to *C. formosanus* in tunneling bioassays with 3-chambered apparatus. Mean ± SD percentage of termite mortality after 10 d

Treatment	Tunneling behavior: % replicates completely penetrated by termites after		% termite mortality
	1 d	5 d	
Control	40	70	2.9 ± 1.6d
Citral	0	60	6.5 ± 6.2cd
Citronellal	20	60	3.6 ± 2.7d
Limonene	30	50	10.4 ± 13.6cd
Eugenol	0	0	19.3 ± 6.3ab
Geraniol	0	0	13.2 ± 2.9abc
Nerol	0	16	24.5 ± 13.3a
Piperidine	16	50	15.3 ± 2.2abc

Sand in the center vial was treated with semiochemicals at a concentration of 0.50 μl/g of sand 24 h before the addition of termites. Means followed by the same letter within columns are not significantly different ($P > 0.05$; Ryan–Einot–Gabriel–Welsch multiple F test [SAS Institute 1987]).

1 d, nor through any of the replicates treated with eugenol or geraniol after 5 d. Significantly greater mortality occurred in replicates treated with eugenol, geraniol, nerol, or piperidine than in the controls after 10 d (Table 3). In the 2nd experiment, where sand was treated 10 d before the termites were introduced, termites did not penetrate through the treated sand in any of the replicates treated with eugenol, geraniol, or nerol after 48 h, demonstrating that the repellent effect of the semiochemicals remained in the sand for at least 12 d (Table 4). Mortality was significantly greater in replicates exposed to eugenol and nerol compared with controls, but mortality among geraniol and piperidine-treated replicates and controls did not differ (Table 4). When termites were placed beneath a layer of eugenol-treated sand in a plastic container, significantly less feeding occurred on wood blocks placed on the surface of the sand. Significantly greater termite mortality was observed at a concentration of 0.2 μl/g of sand, but not 0.16 μl/g of sand (Table 5).

Table 4. Toxicity and repellency of semiochemicals to *C. formosanus* in tunneling bioassays with 3-chambered apparatus. Mean ± SD percentage of termite mortality after 72 h

Treatment	No. replicates completely penetrated by termites after		% termite mortality
	24 h	48 h	
Control	5	7	2.4 ± 1.6c
Eugenol	0	0	22.5 ± 18.3a
Geraniol	0	0	14.7 ± 15.3abc
Nerol	0	0	18.3 ± 21.6ab
Piperidine	3	4	3.8 ± 2.6bc

Sand in the center vial was treated with semiochemicals at a concentration of 1 μl/g of sand 10 d before the addition of termites. Means followed by the same letter within columns are not significantly different ($P > 0.05$; Ryan–Einot–Gabriel–Welsch multiple F test [SAS Institute 1987]).

Table 5. Mean ± SD wood consumed by *C. formosanus* and mean ± SD percentage of mortality after 25 d exposure to eugenol-treated sand in containers filled with a treated and an untreated layer of sand

Treatment sand, μl/g	Wood mass loss, g	% termite mortality
Control	0.50 + 0.27a	10.7 + 2.6a
0.16	0.50 + 0.27a	12.0 + 5.3a
0.20	0.07 + 0.10b	51.5 + 44.3b

Means followed by the same letter within columns are not significantly different ($P > 0.05$; Ryan–Einot–Gabriel–Welsch multiple F test [SAS Institute 1987]).

Fumigant Activity. Fumigant toxicity is a primary cause of mortality to termites exposed to the monoterpenoids citral, geraniol, and eugenol. Eugenol was the most effective as a fumigant. Total mortality was observed when termites were exposed to volatile compounds released from sand treated with 8.9 and 8.8 μg/g citral and geraniol and only 1.0 μg/g of eugenol, whereas a fumigant effect from exposure to geraniol-treated sand at a concentration of 1.0 μg/g of sand was not discerned (Table 6).

Feeding Deterrence Tests. Termites were not deterred by eugenol-treated wood, except at a solution concentration of 100,000 ppm (Table 7). Termites also suffered > 70% mortality when exposed to wood blocks treated with 100,000 ppm of eugenol.

Discussion

Differences in the termiticidal activity of the different natural products tested were significant. With the exception of limonene, the monoterpenes were more toxic to *C. formosanus* than the 2 ant-

Table 6. Mean ± SD percentage of mortality from fumigant activity of monoterpenoids to *C. formosanus* confined to uncovered petri dishes placed in containers on top of treated sand for 48 h

Treatment	Concn of sand, μg/g	% termite mortality
Control		0.0 ± 0.0c
Citral	8.9	100.0 ± 0.0a
	1.0	95.3 ± 7.3a
	0.8	20.6 ± 35.2b
	0.5	4.0 ± 3.7c
	0.1	6.0 ± 2.8bc
Geraniol	8.8	100.0 ± 0.0a
	1.0	2.0 ± 4.5c
	0.1	1.3 ± 1.8c
Eugenol	10.7	100.0 ± 0.0a
	1.0	100.0 ± 0.0a
	0.8	93.3 ± 14.9a
	0.5	34.0 ± 28.4b
	0.1	8.0 ± 4.5bc

Termites had no direct contact with sand. Means followed by the same letter are not significantly different ($P > 0.05$; Ryan–Einot–Gabriel–Welsch multiple F test [SAS Institute 1987]).

Table 7. Mean ± SD wood consumed by *C. formosanus* and mean ± SD percentage of mortality after 20 d feeding on blocks of Douglas-fir wood treated with eugenol in a no-choice test

Treatment, ppm	Wood mass loss, g	% termite mortality
Control	0.56 ± 0.13a	9.6 ± 2.8a
1,000	0.57 ± 0.18a	11.6 ± 8.4a
10,000	0.49 ± 0.15a	22.8 ± 12.2a
100,000	0.07 ± 0.10b	71.1 ± 32.2b

Means followed by the same letter within columns are not significantly different ($P > 0.05$; Ryan–Einot–Gabriel–Welsch multiple F test [SAS Institute 1987]).

derived alkaloids and the hydrocarbon, *n*-undecane. Pyrrolidine was more toxic to termites than were piperidine and *n*-undecane. The monoterpenoid alcohols, particularly eugenol, were the most effective as termiticides to *C. formosanus*. Eugenol also showed greater fumigant toxicity than geraniol and citral.

Eugenol is found in many plants, including clove oil, and is used commercially in perfumes and in flavoring of foods (Lewis 1993). Eugenol caused > 50% mortality of *C. formosanus* at a concentration of only 0.04 μl/g of sand when termites were continuously exposed to treated sand in test tubes. When termites were confined in containers with a treated and an untreated layer of sand, eugenol had a detrimental effect on mortality and feeding at a concentration of 0.20 μl/g of sand. However, eugenol was not an effective feeding deterrent when applied directly to blocks of wood, except at the relatively high concentration of 100,000 ppm. At this concentration, eugenol caused significant mortality of termites in containers even though only the block of wood was treated, presumably because of the toxicity of the vapors as indicated by its fumigant activity (Table 6). Volatility can be beneficial for insecticidal treatments under concrete slabs. For example, ethylene dibromide has been used in the past for subslab treatments against subterranean termites (Ebeling 1975).

Because of its volatility and low toxicity to mammals, eugenol may be a candidate for use as a fumigant treatment against subterranean termites. Fumigant activity of other monoterpenoids has been demonstrated against the red flour beetle, *Tribolium castaneum* (Herbst), and the house fly (Rice and Coats 1994). Although fumigation is primarily used to control drywood termite infestations, the fumigants methyl bromide and sulfuryl fluoride have also been used to control aboveground and shipboard infestations of *C. formosanus* (LaFage et al. 1983, Su and Scheffrahn 1986). Aerial *C. formosanus* colonies with no connection to the ground are not uncommon and can cause significant structural damage. Because of regulatory pressures affecting the use of methyl bromide, development of viable alternatives to conventional fumigants, such as carbon dioxide (Delate et al. 1995), are of interest. Further studies will be necessary to determine the efficacy of monoterpenoids as fumigants against termites.

Previous studies have demonstrated that the ant-derived monoterpenoid dolichodial is both toxic and repellent to the Formosan subterranean termite (Cornelius and Grace 1994, Cornelius et al. 1995). Except for eugenol, the monoterpenoids used in this study are recognized as ant alarm pheromones (Hölldobler and Wilson 1990). Because ants are major enemies of termites, semiochemicals signalling the presence of ants might act as repellents to termites even at sublethal levels. However, when these semiochemicals were present in the sand at nontoxic levels, termites were repelled only for a short period. For example, termites did not penetrate through sand treated with citral for at least 24 h at sublethal concentrations. However, termites had completely penetrated through 60% of citral-treated replicates after 5 d. The ant alarm pheromone *n*-undecane had no effect on either suvival or tunneling behavior of termites.

Monoterpenoids are potentially useful as natural sources for the development of novel insecticides because of their low mammalian toxicity. Other research has shown that different monoterpenoids differ significantly in their toxicity to different insect species (Cook and Hain 1988, Rice and Coats 1994). In our study, the potency of different monoterpenoids to *C. formosanus* varied significantly. Of the semiochemicals that we evaluated, monoterpenoid alcohols were the most toxic to the Formosan subterranean termite.

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