

Toxicity and Repellency of Semiochemicals Extracted from a Dolichoderine Ant (Hymenoptera: Formicidae) to the Formosan Subterranean Termite (Isoptera: Rhinotermitidae)

MARY L. CORNELIUS,¹ J. KENNETH GRACE,¹ PAUL W. FORD,² AND
BRADLEY S. DAVIDSON²

Environ. Entomol. 24(5): 1263-1269 (1995)

ABSTRACT The anal gland secretions of ants in the subfamily Dolichoderinae contain terpenoids that are known to have insecticidal properties. The repellency and toxicity of whole-body extracts and chemical fractions of such extracts from the dolichoderine ant *Ochetellus glaber* (Mayr) to *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) were examined. Extract-treated sand caused significant termite mortality in a direct-exposure assay. Termites were also repelled by extract-treated sand and the chemical repellency lasted for at least one month in treated sand. The principal repellent components of *O. glaber* secretions were determined to be *cis,trans*- and *trans,cis*-isomers of dolichodial in a 3:1 ratio.

KEY WORDS *Coptotermes formosanus*, *Ochetellus glaber*, monoterpenes

SUBTERRANEAN TERMITES ARE the greatest single cause of damage to wooden structures in the United States. It is estimated that damage caused by subterranean termites reaches \$1-\$2 billion annually (Hedges 1992). In Hawaii, estimated costs to control infestations and repair damage caused by the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, exceed \$60 million each year (Yates and Tamashiro 1990). Formosan subterranean termites are also serious pests in the southern gulf states (Su and Scheffrahn 1986), and they have recently been discovered in California (Atkinson et al. 1993).

There has been great interest in the development of naturally produced chemicals as alternatives to synthetic insecticides for use in pest control programs. The search for natural products has focused primarily on plant allelochemicals such as azadirachtin (Schmutterer 1990, Scheffrahn 1991, Grace and Yates 1992); however, insects also produce an arsenal of chemical weapons that could be potentially useful in pest control (Blum 1981). For example, ants produce compounds that repel other ant species (Scheffrahn et al. 1984, Andersen et al. 1991). Allelochemicals were effective in disrupting foraging by 2 ant species, *Linepithema humile* (Mayr) (Shorey et al. 1992) and *Formica aerata* (Francoeur) (Shorey et al. 1993).

Although certain ant-produced chemicals are known to be toxic to termites in topical applications (Clement et al. 1986, 1988; Escoubas and Blum 1990), little attention has been given to

their potential as tools for the management of termite populations. The anal gland secretions of ants in the subfamily Dolichoderinae contain terpenoids that are known to have insecticidal properties (Cavill and Houghton 1974, Cavill et al. 1976, 1982). We examined the toxicity and repellency of secretions of the dolichoderine ant *Ochetellus glaber* (Mayr), formerly *Iridomyrmex*, in treated sand.

In previous research, we found that termites were prevented from tunneling through sand treated with *O. glaber* extracts in an indirect-exposure tunneling assay (Cornelius and Grace 1994). In this assay, termites were placed in a container filled with untreated sand and were allowed access to a tunneling arena filled with extract-treated sand. Because termites were repelled by contact with treated sand, they generally remained in the container with untreated sand and did not penetrate through the treated sand in the arena. Therefore, termite exposure to treated sand was limited and there were no effects on termite mortality in this indirect-exposure assay. We examined the effect of extract-treated sand on termite mortality in a direct-exposure assay where termites were confined to containers with treated sand for a continuous exposure. We also conducted experiments to determine the longevity of the repellent effect of the extract on termite tunneling behavior in an indirect-exposure bioassay. The principal repellent components of *O. glaber* secretions were isolated and the identities of the active components as determined using behavioral assays were confirmed by gas chromatography/mass spectrometry and nuclear magnetic resonance techniques.

¹Department of Entomology, University of Hawaii, Honolulu, HI 96822.

²Department of Chemistry, University of Hawaii, Honolulu, HI 96822.

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.

Because *O. glaber* frequently invaded our termite collection traps, ant colonies were readily collected from these traps and maintained in the laboratory at ambient conditions (23–25°C) in uncovered plastic boxes (30 by 16 by 8 cm). The sides of the boxes were coated with liquid teflon (Fluon, Northern Products, Woonsocket, RI), to prevent ants from escaping. Each box contained a 4.5-cm diameter plastic petri dish with a layer of plaster-of-paris in the bottom and a red cellophane-covered lid to provide a suitable nesting site for ants. Ants freely moved through the hole in the lid. They were provided with a constant supply of water from a water-filled, 55-ml polystyrene vial (60 by 35 mm diameter) which 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 plastic Falcon test tube (17 by 100 mm) filled with honey. Freshly killed termites were added to boxes for protein approximately once a week. Ant colonies collected in the field comprised hundreds of workers, several queens, and brood.

Toxicity of Ant Extract in Direct-Exposure Tests. Extracts of *O. glaber* workers were made by soaking ants in dichloromethane for 24 h. Silica sand (Silica 5151 [fine granular silicon dioxide], Fisher Scientific, NJ) was treated with *O. glaber* extract to concentrations of 250, 62.5, 25, 6.25, and 2.5 ant equivalents per gram of sand or with solvent alone. The sand was allowed to dry for 1 h to evaporate the solvent. For each bioassay, 2 g of sand were placed in a plastic Falcon test tube (17 × 100 mm). Sand was moistened with 150 μ l 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, placed in an unlit temperature cabinet (28°C, 80% RH) for 24 h, and termite mortality was recorded. For the 2 lowest concentrations (6.25 and 2.5 ant equivalents), separate series of replicates were also evaluated after 48 h. In each test, there were 10 replicates (30 termites per replicate) of both extract- and solvent-treated sand. Percentage mortality data of were transformed by the arcsine of the square root and compared using a *t*-test (SAS Institute 1987).

Repellency and Longevity of Ant Extract in an Indirect-Exposure Tunneling Bioassay. An indirect exposure tunneling bioassay (Grace 1991a, Grace et al. 1992) was used to evaluate the effect

of "aged" sand treated with ant extract on termite tunneling behavior. Ants were soaked in dichloromethane for 24 h. Two grams of silica sand were treated with 500 μ l of extract (applied by pipette) at concentrations of 250 ant equivalents per gram of sand.

As in our earlier study (Cornelius and Grace 1994), the assay apparatus had 3 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, Guilford, ME) as food; (2) a glass sandwich, or tunneling arena, containing the treated sand, and (3) a 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 2 glass microscope slides (2.5 by 7.5 cm) spaced 3–4 mm apart and secured in a horizontal upright position on a long edge by silicone rubber sealant (General Purpose Clear Sealant, Dow Corning, Midland, MI) to a 3rd flat glass slide as a base. The ends of the tunneling arena were 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 1 of the 2 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 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 and 10 soldiers to approximate natural caste proportions) were placed in one of the adjacent vials. Termites were placed in a vial with untreated sand and allowed access to a tunneling arena filled with treated sand. Therefore, termites were able to avoid continuous, direct exposure to treated sand in this bioassay. In the 1st experiment, the top edge of each tunneling arena was sealed with plaster-of-paris and left at ambient conditions (23–25°C) in the laboratory for 10 d before termites were added. In the 2nd experiment, the extract treated sand was poured into the tunneling arena and left exposed at ambient conditions in the laboratory for 10 d before being sealed with plaster-of-paris. In the 3rd experiment, the top edge of each tunneling arena was sealed with plaster-of-paris and left at ambient conditions in the laboratory for 30 d before termites were added. In each experiment, after termites were added, the vials were capped with plastic lids containing small air holes and the 3-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 was also recorded after 10 d. In each experiment, there were 6 treated and 6 control replicates (100 termites per replicate).

Total tunneling distances in the treated versus control arenas were compared after 5 and 10 d using a *t*-test (SAS Institute 1987). Percentage mortality data after 10 d were transformed by the arcsine of the square root and compared using a *t*-test (SAS Institute 1987).

Chemical Analysis. Ant extracts, obtained by steeping 500 *O. glaber* workers in dichloromethane (1 ml), were subjected to analysis on a HP5989 gas chromatography/mass spectrometry (GC/MS) engine outfitted with a narrow bore 1 μ m DB-1 30 m capillary column (80–23°C at 4°/min). GC injector and detector temperatures were 225 and 250°C, respectively. EI ionization was accomplished at 70 eV and CI at 50 eV with methane as the reagent gas. Fractionation of the crude extract was accomplished by preparative thin layer chromatography (TLC) (Alltech 500 μ m silica gel GF) using hexane-ethyl acetate (2:1) as developing solvent or by using column chromatography over silica gel (Merck) (230–400 mesh) with dichloromethane as eluent. Similar column aliquots, based on analytical TLC analysis and spot visualization with *p*-anisaldehyde spray reagent (Stahl 1969), were combined into 9 fractions labeled A-I. Fractions were reanalyzed using gas chromatography (GC) on a Shimadzu GC-8A gas chromatograph equipped with a wide bore DB-1 capillary column (15 m by 0.53 mm) (80–210°C at 8°/min) and a flame ionization detector (injector and detector temps same as above). Nitrogen (20 ml/min flow rate) was used as carrier gas.

Proton and carbon nuclear magnetic resonance (NMR) spectra were recorded using a GE QE-300 spectrometer operating at 300 MHz for proton and 75 MHz for carbon. Deuteriochloroform was used as solvent.

Behavioral Responses of Termite Soldiers to Chemical Fractions of Ant Extract. Seven chemical fractions and a solvent control were each applied to a termite worker (2 μ l per termite), killed by freezing and washed in dichloromethane. Termite soldiers were used in this bioassay because they were more responsive than workers to differences in extracts of different ant species in our previous research (Cornelius and Grace 1994). Also, we had insufficient sample material to test the responses of both soldiers and workers to fractions. A termite soldier, collected from the same colony as the dead worker, was gently transferred to a 4.5-cm diameter glass petri dish with a wooden stick and allowed to acclimate for 1–2 min. Generally, within 1–2 min, the termite soldier would begin to walk around the edge of the dish. An extract-treated termite worker was then placed along the edge of the petri dish on the opposite side from the soldier. After each trial, the dish was rinsed with methanol and water and dried. For each trial, a new termite soldier and a new extract-treated worker were used. There were 25 replicates for each fraction, except for fraction C, of which there were only 15 replicates because of in-

sufficient sample material. The behavioral responses of termite soldiers to chemical fractions were recorded as described below.

Soldiers of *C. formosanus* showed 4 distinct responses which were scored as follows: (1) *Avoid*: Soldier avoided contact with the termite worker by backing up and walking in the opposite direction or by walking around it without making contact; (2) *No response*: Soldier showed no response after making tactile contact with the test insect. Contact with the test insect did not elicit either an aggressive or alarmed behavioral response; (3) *Open mandibles*: Soldier opened its mandibles after making tactile contact with the test insect; and (4) *Attack*: Soldier attacked the test insect by snapping at it or grabbing it in its mandibles.

For each trial, observations were made on the first 2 encounters and the encounter eliciting the highest score was recorded. Thus, termites had to avoid contact with the test insect in both encounters in order for the interaction to be scored as avoidance and termites had to encounter the test insect twice without eliciting any aggressive response in order for the interaction to be scored as no response.

Repellency of Active Chemical Fraction to Termite Workers. The chemical fraction (E) containing the *cis,trans*- and *trans,cis*-isomers of dolichodial was isolated as described above. Silica sand (Silica 5151 [fine granular silicon dioxide], Fisher, NJ) was treated with fraction E to concentrations of 3.12, 6.25, 12.5, 25, 50, and 100 μ g per gram of sand, or with dichloromethane alone and allowed to dry for 1 h to evaporate the solvent. One gram of sand was placed in a plastic Falcon test tube (12 \times 75 mm). Sand was moistened with 50 μ l of distilled water and 30 *C. formosanus* workers were placed in each test tube. The tubes were capped and placed in an unlit temperature cabinet (28°C, 80% RH) for 72 h. There were 20 replicates (30 termites per replicate) of the following concentrations: 3.12, 6.25, 12.5, and the solvent-treated controls. There were 10 replicates of the 3 highest concentrations, 25, 50, and 100 μ g per gram of sand. Termite penetration into the sand was measured daily. Termite mortality after 72 h was also recorded. Percentage mortality data after 72 h were transformed by the arcsine of the square root and compared using an analysis of variance (ANOVA) (SAS Institute 1987).

Results

Toxicity of Ant Extract in Direct-Exposure Tests. At concentrations of 250 and 62.5 ant equivalents per gram of sand, there was 100% mortality in all replicates after 24 h (Table 1). At a concentration of 25 ant equivalents, mortality was significantly greater than in controls ($P < 0.0001$) and termites that were still alive were immobilized. At concentrations of 6.25 and 2.5 ant equivalents, there was no difference in mortality between treat-

Table 1. Toxicity of *O. glaber* extract-treated sand to *C. formosanus* workers after 24 or 48 h exposures

Extract concentration ^a	Length of exposure	% mean mortality ^b		<i>P</i> > <i>t</i>
		Treatment	Control	
250	24 h	100.0 ± 0.0	2.0 ± 0.74	0.0001 ^c
62.5	24 h	100.0 ± 0.0	0.3 ± 0.33	0.0001 ^c
25	24 h	77.6 ± 8.50	1.3 ± 0.88	0.0001 ^c
6.25	24 h	1.3 ± 0.54	1.0 ± 0.50	0.66
6.25	48 h	12.3 ± 3.40	4.0 ± 0.97	0.04 ^c
2.5	24 h	1.0 ± 0.50	1.6 ± 0.89	0.52
2.5	48 h	5.3 ± 3.22	4.0 ± 1.29	0.70

^a Ant equivalents per gram of sand.

^b Mean ± SEM.

^c Differences between treatments and controls are significant at *P* ≤ 0.05 (*t* test).

ment and controls after 24 h. However, there was a significant difference between treatment and control mortality for the 6.25 concentration after 48 h (Table 1). Termites in treatment tubes appeared to be slower and less active than controls after 24 h. Also, extract-treated sand appeared to have a repellent effect at all concentrations tested. Termites in treatment tubes remained on top of the sand and there was no evidence of tunneling into treated sand, whereas termites readily tunneled into solvent-treated (control) sand.

Repellency and Longevity of Ant Extract in Indirect-Exposure Tunneling Bioassays. In all 3 tunneling experiments, termites were repelled by extract-treated sand for at least 5 d. However, there was no significant difference in the distance tunneled by termites through extract-treated or solvent-treated sand after 10 d (Table 2). Although termites were initially repelled by treated sand, there were no significant differences in mortality between treatment and control replicates (Table 2).

Identification of Extract Components. Gas chromatographic analysis indicated that the crude whole-body extracts of *O. glaber* contained 2 major components in ≈3:1 ratio, which were shown to be isomeric by GC/MS. Both components showed a MH⁺ ion of *m/z* 167 using the CIMS, while exhibiting almost identical fragmentation patterns in the EIMS, although the major isomer showed an M⁺ ion at *m/z* 166, while the minor isomer showed a stronger (M-1)⁺ ion at *m/z* 165.

Initial purification of a crude 500 ant extract using preparative TLC provided an active fraction (E) which contained only the more abundant compound observed by GC (Table 3). The ¹H NMR spectrum included signals for 14 protons, while the proton decoupled ¹³C NMR spectrum contained 10 signals (203.4, 194.6, 149.8, 135.0, 60.1, 40.0, 34.4, 33.5, 30.1, and 20.5 ppm), consistent with a monoterpene structure having a molecular formula of C₁₀H₁₄O₂. The existence of ¹H and ¹³C NMR signals for 2 aldehydes (9.54[s] and 9.40 [d, *J* = 2.7 Hz]; 194.6 ppm and 203.4 ppm, respectively), a terminal alkene (6.27 [d, *J* = 1.45 Hz] and 6.11

Table 2. Mean distance ± SEM penetrated by *C. formosanus* workers through sand in a 7.5-cm long tunneling arena treated with a dichloromethane extract of *O. glaber* at a concentration of 250 ant equivalents per gram of sand

Experiment	Distance penetrated, cm		% mean mortality
	Day 5	Day 10	
10-d delay 1 ^a			
Treatment	2.5 ± 0.6 ^d	5.5 ± 0.8	19.8 ± 4.9
Control	7.5 ± 0.0 ^d	7.5 ± 0.0	8.6 ± 2.7
10-d delay 2 ^b			
Treatment	3.2 ± 1.0 ^d	5.3 ± 2.5	12.0 ± 1.0
Control	7.5 ± 0.0 ^d	7.5 ± 0.0	10.5 ± 2.4
30-d delay ^c			
Treatment	1.6 ± 0.5 ^d	5.6 ± 0.9	12.1 ± 1.4
Control	7.5 ± 0.0 ^d	7.5 ± 0.0	12.5 ± 1.8

^a Extract-treated sand was poured into the tunneling arena and the top edge of each tunneling arena was sealed with plaster-of-paris and left for 10 d before termites were added.

^b Extract-treated sand was poured into the tunneling arena. After 10 d, the top edge of each tunneling arena was sealed with plaster-of-paris and then termites were added.

^c Extract-treated sand was poured into the tunneling arena and the top edge of each tunneling arena was sealed with plaster-of-paris and left for 30 d before termites were added.

^d Differences between treatments and controls are significant at *P* ≤ 0.05 (*t*-test).

[s]; 135.0 and 149.8 ppm], and a secondary methyl group (1.08 [d, *J* = 7.0 Hz] ppm) match that reported in the literature for the cyclopentanoid monoterpene *cis,trans*-dolichodial (Cavill and Hinterberger 1960, 1961, Pagnoni et al. 1976).

A 2nd dichloromethane ant extract was purified using column chromatography over silica gel. A single active fraction (E) was obtained; however, instead of containing a single compound, *cis,trans*-dolichodial was obtained together with the 2nd compound observed in the GC/MS analysis as a 3:1 mixture. The ¹H NMR of the mixture indicated that the isomeric metabolite contained all of the same structural units as dolichodial, including 2 aldehydes (9.73 [d, *J* = 3.0 Hz] and 9.49 [s] ppm), a disubstituted terminal alkene (6.29 and 6.00 ppm), and a secondary methyl group (1.03 [d, *J* = 7.2 Hz] ppm), allowing it to be identified as

Table 3. Percentage of *C. formosanus* soldiers responding to TLC fractions of *O. glaber* extract applied to dead *C. formosanus* workers (2 μl per insect) (25 replicates per fraction)

Fraction	% responses			
	Avoid	No response	Open mandibles	Attack
Solvent	4	60	24	12
A	16	24	36	24
B	8	12	60	20
C	53	0	27	20
D	16	40	32	12
E	92	8	0	0
F	16	8	28	48
G	8	12	32	48

Table 4. Percentage of replicates in which termites penetrated into sand treated with different concentrations of active TLC fraction (E) of *O. glaber* extract

Concentration ^a	% replicates penetrated by termites			% mean mortality ^b
	24 h	48 h	72 h	
Control (20)	100	100	100	1.8 ± 2.8
3.12 (20)	100	100	100	0.6 ± 7.8
6.25 (20)	75	100	100	2.1 ± 2.9
12.5 (20)	80	100	100	1.1 ± 2.4
25 (10)	50	100	100	0.6 ± 7.8
50 (10)	0	30	40	1.3 ± 1.7
100 (10)	0	20	40	4.6 ± 3.9

^a μg of fraction E per gram of sand.

^b Mean \pm SD percentage mortality after 72 h. Means are not significantly different ($P = 0.05$). ($F = 1.27$, $df = 6$, $P = 0.27$; ANOVA).

trans,cis-dolichodial (Cavill and Hinterberger 1960, 1961, Pagnoni et al. 1976).

Behavioral Responses of Termite Soldiers to Chemical Fractions of Ant Extract. Soldiers avoided contact with workers treated with TLC fraction E in 92% of trials, indicating that this fraction contained a repellent compound (Table 3). However, soldiers also avoided contact with workers treated with fraction C in 53% of replicates. Hence, there may be other components of the ant extract contributing to its repellency to termites. Also, termite soldiers attacked workers treated with fractions F and G in 48% of replicates. There were significantly more attacks on workers treated with these 2 fractions than on the solvent control ($\chi^2 = 7.7$, $df = 1$, $P = 0.005$), indicating that these fractions could potentially contain components which elicit an aggressive response from termite soldiers.

Repellency of Active Chemical Fraction to Termite Workers. After 24 h, termites remained on top of the sand and did not tunnel into the sand in any of the replicates treated with 50 or 100 μg of fraction E, and termites tunneled into the sand in only 5 out of 10 replicates treated with 25 μg of fraction E per gram of sand (Table 4). After 48 h, termites tunneled into the sand in all replicates, except for those treated with either 50 or 100 μg of fraction E, in which less than 50% of replicates were penetrated. There were no differences in termite mortality among the different treatments after 72 h (Table 4).

Discussion

The use of monoterpenes for chemical defense is a relatively common strategy among certain groups of insects. Both the *cis,trans*- and *trans,cis*-isomers of dolichodial have been previously isolated as the components of the whole body extracts of a number of *Dolichoderus* and *Iridomyrmex* species of ants (Cavill and Hinterberger 1960), often along with other monoterpenes such as iridomyrmecin and iridodial. *Cis,trans*- and *trans,cis*-

dolichodials have also been isolated from the plant *Teucrium marum* (Pagnoni et al. 1976) and the *trans,trans*-isomer was isolated from the Argentine ant *Linepithema humile* (Cavill et al. 1976). Furthermore, a compound named anisomorphal, which is thought to be identical to *trans,cis*-dolichodial (Pagnoni et al. 1976), was isolated from a stick insect *Anisomorpha buprestoides* (Meinwald et al. 1962). Although the chemistry of *O. glaber* is not novel, it is interesting that the *cis,trans*-isomer is typically found in greater abundance than the *trans,cis*-isomer, although the latter is apparently favored thermodynamically, as demonstrated by base catalyzed equilibrium (Pagnoni et al. 1976).

Dolichoderine ants most likely secrete terpenes as a defense against other ant species (Blum 1981). *Cis,trans*-iridodial, isolated from the dolichoderine *Forelius foetidus* (Buckley), acts as a repellent to 2 other ant species, *Solenopsis maniosa* and *Crematogaster californica* (Scheffrahn et al. 1984). Results of this study demonstrate that the *cis,trans*- and *trans,cis*-isomers of dolichodial are repellent to *C. formosanus*. Termites did not tunnel into sand treated with 50 or 100 μg of dolichodial at all for 24 h and termites in only 40% of replicates tunneled into sand after 72 h. Even after a 30-d delay, termites tunneled a significantly shorter distance through extract-treated sand than through solvent-treated sand over a 5-d period. In previous research, we found that termites tunneled a significantly shorter distance through extract-treated sand at the same concentration for at least 10 d in the absence of a delay between treatment and the addition of termites (Cornelius and Grace 1994), indicating that the repellent component of the extract had undergone some degree of volatilization or degradation during the delay. Termites also appear to habituate to the extract since they tunnel a greater distance each day of their exposure.

Termites that were continuously exposed to extract-treated sand suffered significantly greater mortality than controls; however, extract-treated sand had no effect on mortality in the indirect-exposure bioassays (Cornelius and Grace 1994). In these tests, we observed that termites generally only contacted extract-treated sand briefly and then returned to the vial containing untreated sand. Therefore, direct exposure to ant semi-chemicals was quite limited. Termites only suffered mortality when they were confined in containers with relatively high concentrations of ant extract.

The toxicity and repellency of *O. glaber* semi-chemicals to subterranean termites suggest that ant compounds could potentially be useful in termite control. Repellency is considered to be an important characteristic of soil insecticides and the ability of termites to tunnel through treated soil is evaluated when determining the efficacy of termiticides (Su et al. 1993). In the direct-exposure tests, termites avoided tunneling through dolicho-

dial-treated sand even at the lowest concentration (2.5 ant equivalents per gram of sand). Chemical stability is also very important and the results of this study demonstrate that the repellent component of *O. glaber* extract will remain active in sand for at least a month. However, it would be necessary to develop more stable analogs of dolichodial or formulate it in microencapsulation in order for it to be useful in soil applications.

Recent research has shown that semiochemicals could be used in pest control programs to disrupt the foraging behavior of ants which are tending homopterans in citrus groves (Shorey et al. 1992, 1993). Chemical cues are important in termite communication and influence a wide variety of behaviors such as species, and possibly colony recognition (Howard et al. 1982, Haverty and Thorne 1989, Bagnères et al. 1991, Grace 1991b). Thus, new compounds, modeled after ant semiochemicals, might be used as insecticides or to prevent termite penetration of structures by disrupting the foraging of these destructive insects.

Acknowledgments

We are grateful to R. T. Yamamoto and C. Tome for assistance with termite and ant collections, A. Harmon (McCormick, Hunt Valley, MD) for performing the GC/MS analysis, and to N. Reimer (Hawaii Department of Agriculture, Honolulu, HI) for identification of ant species. Funding was provided by USDA Specific Cooperative Agreement 58-6615-4-037. This is Journal Series No. 4034 of the Hawaii Institute of Tropical Agriculture and Human Resources.

References Cited

- Andersen, A. N., M. S. Blum, and T. H. Jones. 1991. Venom alkaloids in *Monomorium "rothsteini"* Forel repel other ants: is this the secret to success of *Monomorium* in Australian ant communities? *Oecologia* 88: 157-160.
- Atkinson, T. H., M. K. Rust, and J. L. Smith. 1993. The Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae), established in California. *Pan-Pac. Entomol.* 69: 111.
- Bagnères, A.-G., A. Killian, J.-L. Clement, and C. Lange. 1991. Interspecific recognition among termites of the genus *Reticulitermes*: evidence for a role for the cuticular hydrocarbons. *J. Chem. Ecol.* 17: 2397-2420.
- Blum, M. S. 1981. Chemical defenses of arthropods. Academic, New York.
- Cavill, G.W.K. and E. Hinterberger. 1960. The chemistry of ants IV. Terpenoid constituents of some *Dolichoderus* and *Iridomyrmex* species. *Aust. J. Chem.* 13: 514-519.
1961. The chemistry of ants V. Structure and reaction of dolichodial. *Aust. J. Chem.* 14: 143-149.
- Cavill, G.W.K. and E. Houghton. 1974. Volatile constituents of the Argentine ant, *Iridomyrmex humilis*. *J. Insect Physiol.* 20: 2049-2059.
- Cavill, G.W.K., E. Houghton, F. J. McDonald, and P. J. Williams. 1976. Isolation and characterization of dolichodial and related compounds from the Argentine ant *Iridomyrmex humilis*. *Insect Biochem.* 6: 483-490.
- Cavill, G.W.K., P. L. Robertson, J. J. Brophy, D. V. Clark, R. Duke, C. J. Orton, and W. D. Plant. 1982. Defensive and other secretions of the Australian cocktail ant, *Iridomyrmex nitidiceps*. *Tetrahed. Lett.* 38: 1931-1938.
- Clement, J.-L., M. Lemaire, and C. Lange. 1986. Toxicité à l'égard des Termites du genre *Reticulitermes* des Pyrrolidines et Pyrrolines de la grande a poison de *Monomorium minutum*. *C.R. Acad. Sci. Paris* 303(III) 669-672.
- Clement, J.-L., M. Lemaire, P. Nagnan, P. Escoubas, A.-G. Bagnères, and C. Joulie. 1988. Chemical ecology of European termites of the genus *Reticulitermes*. Allomones, pheromones, and kairomones. *Sociobiology* 14: 165-174.
- Cornelius, M. L. and J. K. Grace. 1994. Behavioral responses of the Formosan subterranean termite (Isoptera: Rhinotermitidae) to semiochemicals of seven ant species. *Environ. Entomol.* 23: 1524-1528.
1994. Semiochemicals extracted from a dolichoderine ant affect the feeding and tunneling behavior of the Formosan subterranean termite (Isoptera: Rhinotermitidae). *J. Econ. Entomol.* 87: 705-708.
- Escoubas, P. and M. S. Blum. 1990. The biological activities of ant-derived alkaloids, pp. 482-489. *In* R. K. Vander Meer, K. Jaffe, and A. Cedenio [eds.]. *Applied Myrmecology*. Westview, San Francisco.
- Grace, J. K. 1991a. Responses of eastern and Formosan subterranean termites (Isoptera: Rhinotermitidae) to borate dust and soil treatments. *J. Econ. Entomol.* 84: 1753-1757.
- 1991b. Semiochemical mediation and manipulation of *Reticulitermes* behavior (Isoptera: Rhinotermitidae). *Sociobiology* 19: 147-162.
- Grace, J. K. and J. R. Yates. 1992. Behavioural effects of a neem insecticide on *Coptotermes formosanus* (Isoptera: Rhinotermitidae). *Trop. Pest Management* 38: 176-180.
- Grace, J. K., R. T. Yamamoto, and R. H. Ebesu. 1992. Laboratory evaluation of the novel soil insecticide silafluofen against *Coptotermes formosanus* Shiraki (Isopt., Rhinotermitidae). *J. Appl. Entomol.* 113: 466-471.
- Haverty, M. I. and B. L. Thorne. 1989. Agonistic behavior correlated with hydrocarbon phenotypes in dampwood termites, *Zootermopsis* (Isoptera: Termitidae). *J. Insect Beh.* 2: 523-543.
- Hedges, S. 1992. Termite inspections. *Pest Control Technol.* 20: 30-33.
- Howard, R. W., C. A. McDaniel, D. R. Nelson, G. J. Blomquist, L. T. Gelbaum, and L. H. Zalkow. 1982. Cuticular hydrocarbons of *Reticulitermes virginicus* and their role as potential species and caste-recognition cues. *J. Chem. Ecol.* 8: 1227-1239.
- Meinwald, J., M. S. Chadha, J. J. Hurst, and T. Eisner. 1962. Defense mechanisms of arthropods IX, the secretion of a phasmid insect. *Tetrahed. Lett.* 1: 29-33.
- Pagnoni, U. M., A. Pinetti, R. Trave, and L. Garanti. 1976. Monoterpenes of *Teucrium marum*. *Aust. J. Chem.* 29: 1375-1381.
- SAS Institute. 1987. SAS/STAT Guide for Personal Computers, version 6 ed. SAS Institute, Cary, NC.
- Scheffrahn, R. H. 1991. Allelochemical resistance of wood to termites. *Sociobiology* 19: 257-281.

- Scheffrahn, R. H., L. A. Gaston, J. J. Sims, and M. K. Rust. 1984.** Defensive ecology of *Forelius foetidus* and its chemosystematic relationship to *F.* (= *Iridomyrmex*) *pruinosis* (Hymenoptera: Formicidae: Dolichoderinae). *Environ. Entomol.* 13: 1502-1506.
- Schmutterer, H. 1990.** Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. *Annu. Rev. Entomol.* 35: 271-297.
- Shorey, H. H., L. K. Gaston, R. G. Gerber, P. A. Phillips, and D. L. Wood. 1992.** Disruption of foraging by Argentine ants, *Iridomyrmex humilis* (Mayr) (Hymenoptera: Formicidae), in citrus trees through the use of semiochemicals and related chemicals. *J. Chem. Ecol.* 18: 2131-2142.
- Shorey, H. H., L. K. Gaston, R. G. Gerber, C. B. Sisk, and D. L. Wood. 1993.** Disruption of foraging by *Formica aerata* (Hymenoptera: Formicidae) through the use of semiochemicals and related chemicals. *Environ. Entomol.* 22: 920-924.
- Stahl, E. 1969.** *Thin Layer Chromatography*. Springer, New York.
- Su, N.-Y. and R. H. Scheffrahn. 1986.** The Formosan subterranean termite, *Coptotermes formosanus* (Isoptera: Rhinotermitidae), in the United States: 1907-1985, pp. 31-38. *In* Proceedings, National Conference on Urban Entomology. University of Maryland, College Park.
- Su, N.-Y., R. H. Scheffrahn, and P. M. Ban. 1993.** Barrier efficacy of pyrethroid and organophosphate formulations against subterranean termites (Isoptera: Rhinotermitidae). *J. Econ. Entomol.* 86: 772-776.
- Tamashiro, M., J. K. Fujii, J. K., and P.-Y. Lai. 1973.** A simple method to observe, trap, and prepare large numbers of subterranean termites for laboratory and field experiments. *Environ. Entomol.* 2: 721-722.
- Yates, J. R. and M. Tamashiro. 1990.** The Formosan subterranean termite in Hawaii. Hawaii Institute of Tropical Agriculture and Human Resources Research Extension Series 117. University of Hawaii, Honolulu.

Received for publication 4 November 1994; accepted 30 March 1995.
