Isolation and Identification of 2-Phenoxyethanol from a Ballpoint Pen Ink as a Trail-Following Substance of *Coptotermes formosanus* Shiraki and *Reticulitermes* sp.[1]

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Abstract Isolation and identification of 2-phenoxyethanol from a ballpoint pen ink as a termite trail-following substance was accomplished by using trail-following bioassays, column chromatography, high performance liquid chromatography, and gas chromatography-mass spectrometry. This chemical elicited trail-following in both *C. formosanus* Shiraki and *Reticulitermes* sp.

Key Words 2-phenoxyethanol, *Coptotermes formosanus, Reticulitermes* sp., trail-following substance, chemical ecology.

The introduced Formosan subterranean termite, *Coptotermes formosanus* Shiraki, and other subterranean termites in the genus *Reticulitermes* are strong trail-followers when foraging (Matsumura et al. 1968, 1969, Runcie 1987, Tokoro et al. 1989). Trail pheromones used in foraging play an important role in the organization and maintenance of termite societies (Howse 1984). Tokoro et al. (1989) isolated a trail pheromone from *C. formosanus* and identified it as (Z,Z,E)-3,6,8-dodecatrien-1-ol (DTE-OH), which also was isolated and identified as a trail-following pheromone for *R. virginicus* (Banks) and *R. santonensis* Feytaud (Matsumura et al. 1968, Laduguie et al. 1994). This compound also elicited trail-following behavior of other *Reticulitermes* species (Matsumura et al. 1972).

Termites also follow some non-pheromone chemicals (Becker and Mannesmann 1968, Karlson et al. 1968, Tai et al. 1971, Matsumura et al. 1969, Watanabe and Casida 1963, Birch et al. 1970, Prestwich et al. 1984). Trail-following compounds that are easily synthesized and stable provide potential new tools and control methods for the manipulation of termite foraging in the field.

The trail-following activities of several synthesized (Z)-4-phenyl-3-buten-1-ol derivatives were tested on five species of subterranean termites in the genera of *Coptotermes, Reticulitermes,* and *Schedorhinotermes* (Prestwich et al. 1984). Several glycol compounds isolated from ink pens also were reported to act as termite trail following substance (Becker and Mannesmann 1968). Papermate® (The Gilette Company, Boston, MA) ballpoint pen ink is known to elicit trail following behavior in introduced Formosan subterranean termites and native subterranean termites, but

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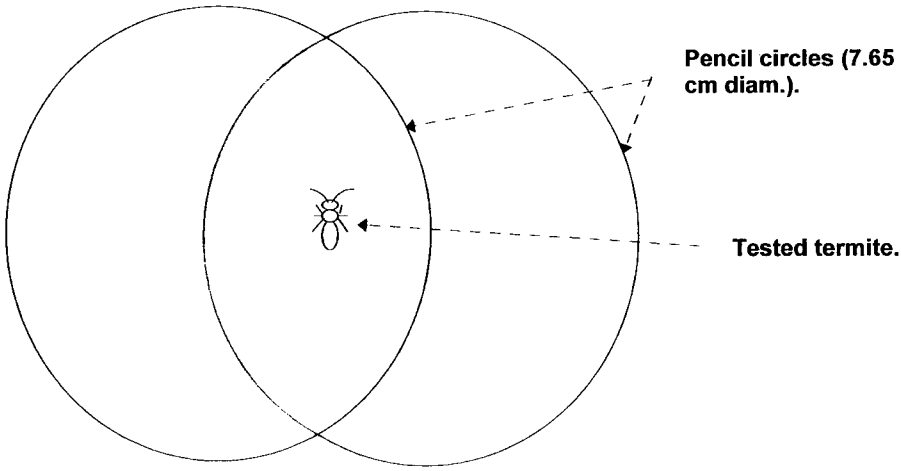

Fig. 1. Trail-following bioassay arena.

the active ingredient(s) have yet to be identified. In this paper, the isolation and identification of the trail-following substance, 2-phenoxyethanol, from Papermate ballpoint pen ink is discussed.

Materials and Methods

Termites. *Coptotermes formosanus* were collected from a home in New Orleans, LA. *Reticulitermes* sp. were collected from cardboard traps in Baton Rouge, LA. Collected termites were kept at room temperature (23 to 28°C) in plastic containers (20 cm diam, 20 cm height) with sand (#4 blasting sand) and moistened corrugated cardboard until testing.

Extraction and isolation. Ink from 30 blue ink Papermate ballpoint pens was dissolved in 200 ml 5% ethanol in water. The ink solution was extracted with 200 ml hexane three times and the upper layer collected, filtered through a filter paper (Whatman 1, qualitative, 15.0 cm), and concentrated to 5 ml under reduced pressure. The extract was chromatographed on a silica gel glass column (5 cm long, and 2 cm ID) packed with silica gel (70-230 mesh, 60 Å average pore diam, SIGMA Chemical Co., St. Louis, MO) and successively eluted with 100 ml hexane and 400 ml 50% ethyl acetate in hexane. The ethyl acetate-hexane elution fraction was collected and concentrated to 2 ml under reduced pressure. This fraction was further fractionated by high-performance liquid chromatography (HPLC) equipped with a normal-phase SUPELCOSIL™ LC-Si column (25 cm × 4.6 mm, 5 µl particle size and 100 Å pore size, SUPELCOL). Elution was performed with hexane-dichloromethane in gradient mode at a flow rate of 1.0 ml/min. The solvent composition was programmed as follows: 100% hexane for the first 30 min, then a 30 min linear gradient from 100% hexane to hexane-dichloromethane 95:5%, followed by a linear gradient in 10 min from hexane-dichloromethane 95:5% to hexane-dichloromethane 70:30%, then another linear gradient in 5 min from hexane-dichloromethane 70:30% to 100% dichloromethane, fol-

Table 1. Results of bioassay for HPLC fractions of blue ink (standard error in parenthesis)

Fraction number	Mean points*
1 to 33	0.0 (0.0)
34	4.6 (0.6)
35	16.1 (2.8)
36	20.0 (2.6)
37	16.9 (3.2)
38	22.8 (5.6)
39	18.7 (3.5)
40	18.8 (2.8)

* One point was given for each continuous 3 cm traveled by a termite over a one-minute period.

lowed by dichloromethane for 5 min. The column was flushed with 100% dichloromethane for 20 min after each run (total about 200). One fraction was collected every 2 min (total 40). Each fraction was concentrated into 0.3 ml under a flow of nitrogen.

Instruments. HPLC was conducted with a Ranin Rabbit HP/HPX 2 pump Solvent Delivery System (10 ml). A Knauer Variable Wavelength Monitor was used as a detector. The GC-MS used was a Hewlett Packard 5890 Series II gas chromatograph equipped with a capillary DB-5 column and coupled with a Hewlett Packard 5971A mass selective detector. The injection temperature was 250°C. The oven temperature was kept at 50°C for the first 3 min, programmed 20°C min^{-1} to 280°C, and held for 6.5 min. Helium carrier gas was delivered at a velocity of approximately 40 cm/sec. The ion source temperature was 200°C, and the ionization voltage was 70 eV.

Screening bioassay for HPLC fractions. Only *C. formosanus* workers were used in the initial screening. The trail-following bioassay was conducted on two overlapped pencil circles (Fig. 1). Each sample was streaked along one of the circles with a 4 µl-micropipette on nonabsorptive paper. A solvent (control) was streaked along the overlapping circle. After evaporation of the solvent (1.5 min), a termite was placed in the center of the overlapping circle. The arena was covered with a red plastic container to reduce visible light and extraneous air flow. To score trail following activity, one point was given for each continuous 3 cm traveled by a termite over a 1-min period. Three replications were performed for each fraction. A new termite was used, and new circles were drawn for each trial. A fraction was considered not active if no points were assigned; it was considered moderately active if, on average, a termite followed the circle between 3 to 6 cm; it was considered very active if a termite followed the trail for 6 cm or more.

Determination of active ingredient. From the above screening bioassay, we determined the HPLC fractions that showed trail-following activity. Eight fractions, numbers 29 to 36 (numbers refer to time eluted), were selected for further GC-MS analysis to identify the compound(s) responsible for trail-following activity. Comparison of neighboring fractions that covered the transition from no trail-following behavior

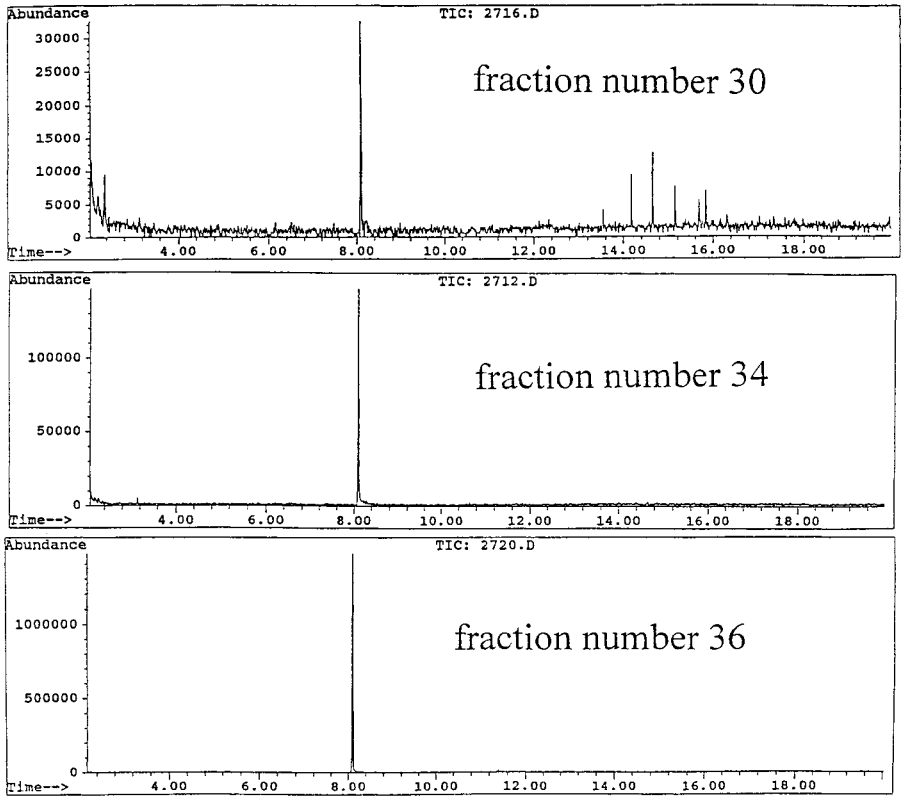

Fig. 2. Total ion chromatograms of HPLC fractions 30, 34, 36.

to high trail-following activity helped identify the active peak(s), therefore, fractions number 29 to 33 which showed no trail-following activity also were subjected to further GC-MS analysis. Identification of the trail-following compound was made by comparison of its GC-retention time and mass spectra with those of authentic compounds.

Bioassay on standard 2-phenoxyethanol. Four concentrations of 2-phenoxyethanol, 0.23, 0.023, 0.0023, 0.00023 μg/cm, were tested. The trail-following bioassay set-up and data collection were similar to those for HPLC fraction screening bioassay (Fig. 1). Each trial was replicated 10 times for both workers and soldiers of both *C. formosanus* and *Reticulitermes* sp.

Results

Screening bioassay for HPLC fractions. Results of the screening bioassay for each HPLC fraction are summarized in Table 1. HPLC fraction number 34 was moderately active. Fractions 35 to 40 were very active.

Determination of active ingredient. One peak with retention time 8.1 min existed in all eight HPLC fractions selected for GC-MS analysis (Fig. 2, HPLC fractions 30,

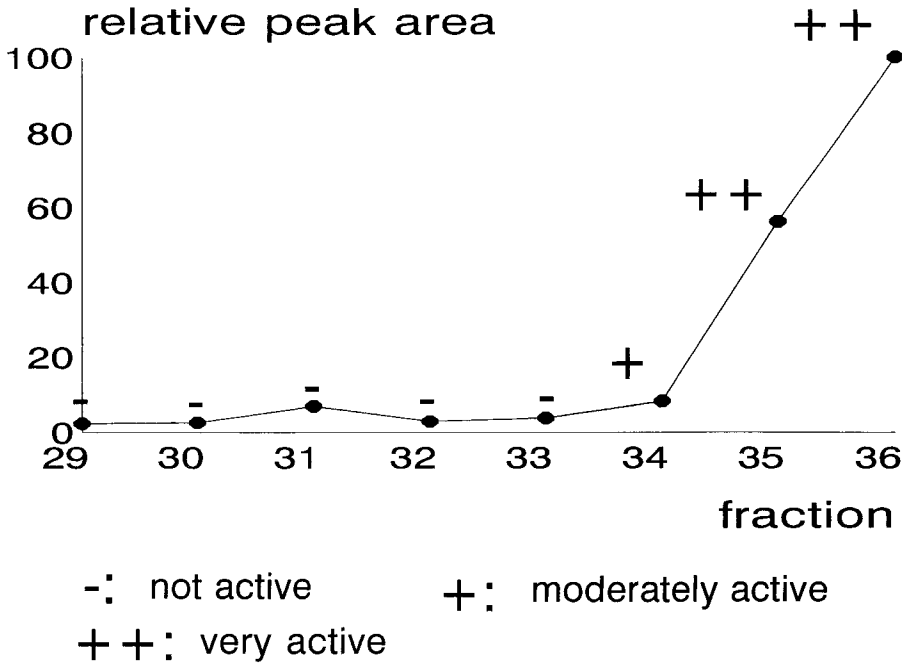

Fig. 3. Relative area of the peaks with retention time 8.10 min. from HPLC fractions 29 to 36.

34, and 36). From fraction number 34 to 36, this peak was dramatically intensified (Fig. 3) which closely paralleled trail-following activity of those fractions. A computer library search and comparison of mass spectra and retention times with a standard compound all confirmed the compound to be 2-phenoxyethanol (Fig. 4). The chemical structures of 2-phenoxyethanol and natural trail pheromone of Formosan subterranean termites are shown in Fig. 5.

Bioassay on standard 2-phenoxyethanol. Authentic 2-phenoxyethanol elicited trail-following behavior to *C. formosanus* workers and soldiers, and *Reticulitermes* sp. workers and soldiers at concentrations 0.23 µg/cm, 0.023 µg/cm, and 0.0023 µg/cm. Limited trail-following activity occurred at the 0.00023 µg/cm (Table 2).

Discussion

Termites exhibit trail-following behavior to a several nonpheromone chemicals (Becker and Mannesmann 1968, Karlson et al. 1968, Tai et al. 1971, Matsumura et al. 1969, Watanabe and Casida 1963, Birch et al. 1970, Prestwich et al. 1984). Prestwich et al. (1984) argued that although receptors for trail pheromones are specific for each termite species, a certain latitude may exist for analogs to elicit a trail-following response. The natural termite trail pheromone of Formosan subterranean termites share little structural similarity to 2-phenoxyethanol except that they are

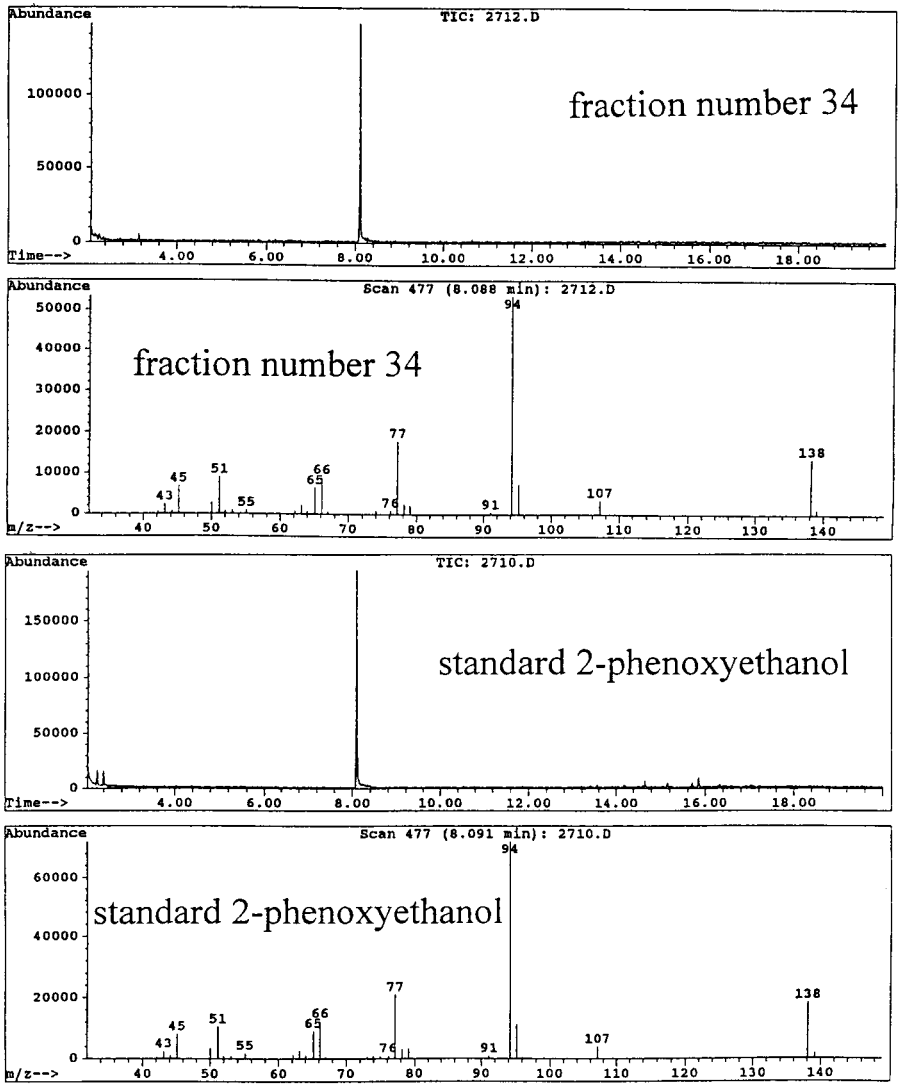

Fig. 4. Chromatograms of fraction 34 and standard 2-phenoxyethanol and mass spectra of the peaks at retention time 8.10 minute.

both primary alcohols. It remains unknown whether this indicates wider latitude tolerance of the receptor site or whether multiple receptors are present.

Termite trail-following to non-pheromone chemicals may cause difficulty in identifying natural termite trail pheromone. In order to distinguish a natural trail pheromone from other trail-following substances, critical diagnostic steps are to: (1) localize the glandular source, (2) extract the active deposition, and (3) compare the stored chemi-

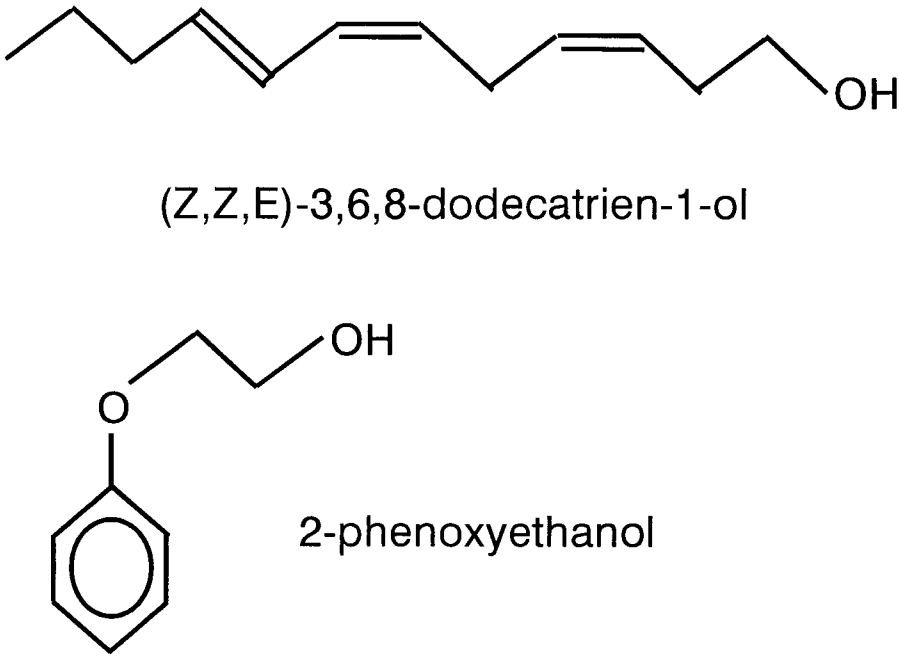

Fig. 5. Chemical structures of natural trail pheromone (Z,Z,E-3,6,8-dodecatrien-1-ol) of Formosan subterranean termites and 2-phenoxyethanol.

cal and the deposited pheromone in both structure and biological activity (Howard et al. 1976). Sampling fresh termite trails may be the only way to confirm the active deposition of the trail pheromone(s) of termites. Although there were examples of successes in other termite species through this process (Tschinkel and Close 1973), similar success for Formosan subterranean termite has eluded us thus far.

Rust et al. (1996) reported that trail-following chemicals from the brown rot fungus, *Gloeophyllum trabeum* (Pers. ex Fr.), stimulated oriented tunneling of *R. hesperus* Banks and argued that application of this chemical in a bait might increase the likelihood for termite workers to encounter the bait. Whether 2-phenoxyethanol can be used to improve termite baits will require an understanding of the interaction between termites in the field and this chemical's effect on several behaviors like orientation, trail-following, and foraging.

Like many other trail-following substances and pheromones, 2-phenoxyethanol is volatile. It remains a challenge to stabilize the chemical long enough to orient foraging termites toward a bait (and away from another food source). Grace (1990) argued that volatility and rapid degradation of trail-following compounds could be overcome by using stable analogues or chemical protectants. Although this direction is being investigated for increasing bait attractiveness, documentation of success has not been reported. Another avenue of research remains in the discovery of more stable chemicals that elicit similar behavior modifying attributes for termites. Our present studies on chemicals naturally associated with Formosan termite colonies are proving fruitful to this end.

Table 2. The response of *Coptotermes formosanus* and *Reticulitermes* sp. to 2-phenoxyethanol trail of different concentrations

Concn. (µg/cm)	Mean points* (standard error in parenthesis)				Percentage of termites following the trail (%)			
	C. formosanus		*Reticulitermes* sp.		*C. formosanus*		*Reticulitermes* sp.	
	Workers	Soldiers	Workers	Soldiers	Workers	Soldiers	Workers	Soldiers
0.23	13.9 (11.2)	18.6 (12.9)	25.9 (9.1)	20.6 (11.9)	75	80	90	70
0.023	15.3 (12.3)	15.5 (12.8)	22.1 (7.4)	20.1 (9.2)	80	75	95	80
0.0023	4.2 (6.2)	4.1 (6.8)	5.6 (8.7)	5.1 (8.3)	65	65	70	60
0.00023	0.5 (1.2)	0.7 (1.4)	0.6 (1.5)	1.7 (5.3)	20	30	25	25

* One point was given for each continuous 3 cm traveled by a termite over a 1-min period.

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References

Becker, V. G. and R. Mannesmann. 1968. Untersuchungen über das verhalten von termiten gegenüber einigen spurbildenen stoffen. Z. Angem. Entomol. 62: 399-436 (in German).

Birch, A. J., K. B. Chamberlain, B. P. Moore and V. H. Powell. 1970. Termite attractants in *Santalum spicatum,* Australian J. Chem. 23: 2337-2341.

Grace, J. K. 1990. Effect of antioxidants on eastern subterranean termite (Isoptera: Rhinotermitidae) orientation to fungal extract. Proc. Entomol. Soc. Wash. 92: 773-777.

Howard, R. W., F. Matsumura and H. C. Coppel. 1976. Trail-following pheromone of the Rhinotermitidae: Approaches to their authentication and specificity. J. Chem. Ecol. 2: 147-166.

Howse, P. E. 1984. Sociochemicals of termites, Pg. 475-519. *In* W. J. Bell and R. T. Carde (eds.), Chemical Ecology of Insects. Chapman and Hall, Sinauer Associates, London.

Karlson, P., M. Lüscher and H. Hummel. 1968. Extraktion und biologische auswertung des spurpheromons der termite *Zootermopsis nevadensis.* J. Insect Physiol. 14: 1763-1771.

Laduguie, N., A. Robert, O. Bonnard, F. Vieau, J.-L. L. Quere, E. Semon and C. Bordereau. 1994. Isolation and identification of (3Z, 6Z, 8E)-3,6,8-dodecatrie-1-ol in *Reticulitermes santonensis* Feytaud (Isoptera, Rhinotermitidae): roles in worker trail-following and in alate sex-attraction behavior. J. Insect Physiol. 40: 781-787.

Matsumura, F., H. C. Coppel and A. Tai. 1968. Isolation and identification of termite trail-following pheromone. Nature 219: 963-964.

Matsumura, F., A. Tai and H. C. Coppel. 1969. Termite trail-following substance, isolation and purification from *Reticulitermes virginicus* and fungus infected wood. J. Econ. Entomol. 62: 599-603.

Matsumura, F., D. M. Jewett and H. C. Coppel. 1972. Interspecific response of termites to synthetic trail-following substance. J. Econ. Entomol. 65: 600-602.

Prestwich, G. D., W.-S. Eng, E. Deaton and D. Wichern. 1984. Structure-activity relationships among aromatic analogs of the trail-following pheromone of subterranean termites. J. Chem. Ecol. 10: 1201-1217.

Runcie, C. D. 1987. Behavioral evidence for multicomponent trail pheromone in the termite, *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae). J. Chem. Ecol. 13: 1967-1978.

Rust M. K., K. Haagsma and J. Nyugen. 1996. Enhancing foraging of western subterranean termites (Isoptera: Rhinotermitidae) in arid environments. Sociobiology 28: 275-286.

Tai, A., F. Matsumura and H. C. Coppel. 1971. Synthetic analogues of the termite trail-following pheromone, structure and biological activity. J. Insect. Physiol. 17: 181-188.

Tokoro, M., M. Takahashi, K. Tsunoda and R. Yamaoka. 1989. Isolation and primary structure of the trail pheromone of the termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) Wood Res. 76: 29-38.

Tschinkwl, W. R. and P. G. Close. 1973. The trail pheromone of the termite *Trinervitermes trinervoides.* J. Insect. Physiol. 19: 707-721.

Watanabe, T. and J. E. Casida. 1963. Response of *Reticulitermes flavipes* to fractions from fungus infected wood and synthetic chemicals. J. Econ. Entomol. **56: 300-307.**