Increased Search Tunnel Formation by *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) in 2-Phenoxyethanol Treated Sand[1]

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Abstract Termites follow chemical and physical gradients in their search for food. In laboratory bioassays, search tunnel formation of *Coptotermes formosanus* Shiraki was examined in response to different 2-phenoxyethanol concentrations in sand. 2-phenoxyethanol significantly increased the total tunnel network length by termites. At a concentration of 0.082%, but not 0.164%, it also significantly increased daily search activity by *C. formosanus*. After aging the treated sand for 2 wks, *C. formosanus* continued to show a significant increase in the search tunnel length in 0.082% 2-phenoxyethanol treatments. Application of 2-phenoxyethanol, a nonpheromone attractant, to termite infested soil nearby, may increase the search and thus the likelihood for contact with baits and nonrepellent termiticides in the field.

Key words Formosan subterranean termite, attractant, trail-following, foraging.

Subterranean termites show trail-following behavior in response to pheromones (Matsumura et al. 1968, 1969, Tokoro et al. 1989, 1994). One active trail compound, (Z, Z, E)-3, 6, 8-dodecatrien-1-ol, has been isolated and identified from body extracts of *Reticulitermes virginicus* (Banks) and *Coptotermes formosanus* Shiraki (Matsumura et al. 1968, 1969, Tokoro et al. 1989). Extracts of the brown rot fungus, *Gloeophyllum trabeum* (Pers. ex Fr.) Murr., contain the same compound and elicit trail-following behavior in several *Reticulitermes* spp. (Smythe et al. 1967, Matsumura et al. 1968, 1972, Grace 1991). Some nonpheromone chemicals, such as several synthesized (Z)-4-phenyl-3-buten-1-ol derivatives and 2-phenoxyethanol, also initiate this behavior in termites (Watanabe and Casida 1963, Tai et al. 1971, Prestwich et al. 1984, Chen et al. 1998). Moreover, trail pheromones induce recruitment and orientation responses in termites (Hall and Traniello 1985, Traniello and Robson 2000).

Foraging in termites begins with the deposition of pheromones released from the sternal gland and, along with other factors, is affected by food source quality and size, and the compass directional aspect of a building as it relates to temperature and

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moisture requirements (Traniello and Robson 1995, 2000, Amburgey and Smythe 1977, Reinhard et al. 1997, Hedlund and Henderson 1999, Su and Puche 2003, Fei and Henderson 2004). Field experiments by Rust et al. (1996) showed that trail-following chemicals from the brown rot fungus *G. trabeum* enhanced oriented tunneling of *R. hesperus* Banks from a distance, suggesting that nonpheromone chemicals increased movement along a gradient and might improve attack percentages of termite baits in the field.

2-Phenoxyethanol was identified from a ballpoint pen ink and shown to be a termite trail-following substance (Chen et al. 1998). 2-Phenoxyethanol is a volatile, nonhazardous organic solvent used in many ballpoint ink formulations (LaPorte et al. 2004). The compound is relatively long lived and ages in a predictable manner over months (Stewart 1985), a factor used to indicate the aging of inks in forensic science (Stewart 1985, LaPorte et al. 2004). In laboratory choice tests with untreated and 2-phenoxyethanol-treated filter paper at 0.012 ~ 1.92%, it also was found to be an attractant to Formosan subterranean termites (Ibrahim et al. 2005).

To further evaluate the potential value of this chemical as an additive to attract termites to baits or into liquid insecticide treatments, we examined its effect on the tunneling behavior by the Formosan subterranean termite. We used a two-dimensional foraging arena to test if 2-phenoxyethanol as an additive could increase search by Formosan subterranean termites in treated sand.

Materials and Methods

Termite. Four *C. formosanus* colonies were collected in New Orleans (colony A, B, and C) and Lake Charles (colony D), LA, by using the milk crate-trapping technique (Smith et al. 2004). Collected termites were returned to the laboratory and maintained in covered, 140-L plastic trash cans until extracted for experiments.

Experimental arena. The experimental arena was similar to that described by Hedlund and Henderson (1999) with some modifications. The experimental apparatus (Fig. 1) consisted of a circular food chamber and a square tunneling chamber that did not contain food. The square tunneling chamber was constructed of two sheets of transparent Perspex® CP acrylic (ANC Plastics, Houston, TX) (33 × 33 × 0.3 cm) separated from each other by four narrow edge lamini (1.3 × 0.25 × 33 cm or 30.5 cm) of transparent Perspex® CP acrylic placed between the outer margins and held together with metal binder clips. Inner dimensions of the tunneling chamber were 30.5 × 30.5 × 0.25 cm. The circular food chamber was a small Petri dish (5.2 cm diam × 3.8 cm) that fit in the center and on top of the square tunneling chamber. The Petri dish was filled with ≈30 cm^3 of #4 blasting sand (Easy Crete, Greenwell Springs, LA) moistened with distilled, deionized water. Small wooden blocks (0.8 × 0.8 cm) were set in the sand with 1 side exposed in the center of the circular chamber. A 0.5-cm-diam hole connected the food and tunneling chambers to allow termites to travel between them. The hole was blocked by transparent tape between the two chambers to prevent termites from entering the tunneling chamber until the trials began. The experimental arena was divided by two 33 × 15.2 × 0.3 cm sand areas and one 33 × 2.6 × 0.3 cm empty space band. The tunneling apparatus was suspended above the floor by glass supports to allow viewing of termite activity in the tunneling chamber from below.

Experimental design. A stock solution of 2-phenoxyethanol was prepared by dissolving 5 ml (=5.51 g [AI]) in 100 ml ethyl alcohol to produce a concentration of

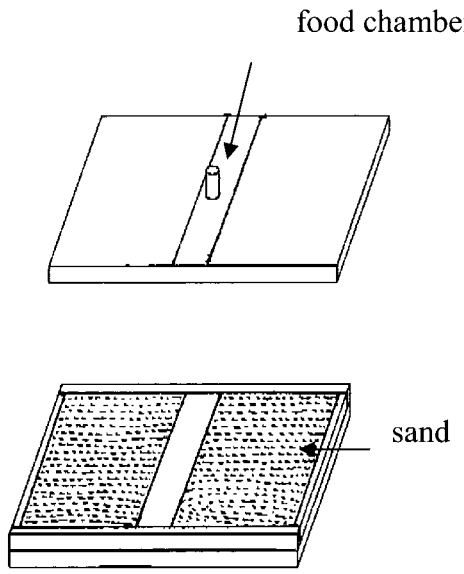

Fig. 1. The experimental apparatus consisted of a circular food chamber and two transparent plastic sheets, separated from each other by four narrow edge lamini between the outer margins.

5% (w/v). Two experiments were conducted to investigate the effect of 2-phenoxyethanol on the tunneling system by Formosan subterranean termites.

In the first experiment (Experiment 1), the three concentrations were: 0.041, 0.082, and 0.164% (w/w of sand). Treated sand at different concentrations (ethyl alcohol only for the control) was dried on plastic trays for 4 h at ambient conditions. For each tested concentration, three experimental apparati (replicates) were used that consisted of six 33 × 15.2 × 0.3 cm bands. In each experimental apparatus, one of the bands (treated side) was filled with 200 cm^3 of #4 blasting sand treated with 2-phenoxyethanol, and another (control side) was filled with sand treated with ethyl alcohol only. The treated band side and the control band side were randomly assigned. Each band of sand was moistened with 50 ml of distilled, deionized water. Termites (n = 150) from colony A (undifferentiated larvae of at least the third instar with 10% soldiers) were released in the center chamber. The experimental apparatus was covered by black paper and maintained at 22-27°C. The same experiment was repeated using termites from colony B.

In the second experiment (Experiment 2), residues of 2-phenoxyethanol on pretreated sand with a concentration of 0.082% (w/w) were tested at 2 wks based on results obtained from experiment 1. Treated sand at 0.082% was placed in a closed glass jar and maintained in the dark at laboratory conditions for 2 wks before testing. The experimental arena and the testing procedure were the same as previously described. Termites from two colonies, colony C and colony D, were used in this experiment.

Measuring tunneling system and data analysis. Termites were acclimated for 24 h in the feeding chamber and then allowed access to the tunneling chamber by

removing the tape in the center chamber. Observations then were recorded every day up to 5 d. Tunnels were traced each day by using different pen colors on the transparent plastic sheet to denote the day of observations. On day 5, the number and the length of tunnels were measured (using a string and ruler), and living termites were counted.

For analysis of tunneling system, there were five dependent measures at five time points (day 1 to day 5). A repeated measures analysis of variance was conducted to determine significant differences in daily search tunnels among treatments with time (SAS Institute 1996). The total length of tunnels between treatments was analyzed using a paired *t*-test (SAS Institute 1996). Mean percent survival of termites at different concentrations was analyzed by an ANOVA using the GLM procedure followed by Tukey's Studentized range test (SAS Institute 1996). To stabilize the variance, data on daily length of tunnels were transformed by log(x) and data on survival rate of termites were arcsine square-root transformed. Data were back-transformed after statistical analyses for presentation.

Results

Search tunnel formation in response to 2-phenoxyethanol concn. 2-Phenoxyethanol at a concentration of 0.082% (w/w) significantly affected daily search tunnels by *C. formosanus* for both colony A ($F = 84.63$; df = 1, 4; $P = 0.0008$) and colony B ($F = 4.55$; df = 1, 4; $P = 0.0295$). At 0.082%, more search tunnels were formed in the 2-phenoxyethanol treated side compared with the control on day 1, day 2, and day 4 by termites from colony A (Table 1). For termites from colony B, mean search length of tunnels was higher in the 2-phenoxyethanol treated side than that in the control on day 1, day 3, and day 4 (Table 1). On day 5, although less tunnels were formed in the 2-phenoxyethanol treated side compared with the control at 0.082% (colony A) and 0.041% (colony B) due to the limitation of foraging areas, much wider tunnels were constructed by termites in the 2-phenoxyethanol treated sides (Table 1). In the 0.082% 2-phenoxyethanol treated side, the total tunnel network length was significantly more extensive compared with the control side for both colony A ($t = -9.93$; $P = 0.010$) and colony B ($t = -7.0$; $P = 0.0198$) (Table 2).

At a concentration of 0.041%, although mean daily search length of tunnels in the 2-phenoxyethanol treated sand was significantly different from the control for termites from colony A ($F = 9.68$; df = 1, 4; $P = 0.0358$), the difference was not significant for termites from colony B ($F = 0.06$; df = 1, 4; $P = 0.8150$) (Table 1). At 0.164%, there was no significant difference of mean daily search length between the treated side and the control for either colony (colony A: $F = 0.01$; df = 1, 4; $P = 0.9151$; colony B: $F = 0.71$; df = 1, 4; $P = 0.4478$) (Table 1). The total tunnel network length was not significantly different between the 2-phenoxyethanol treated side and the control at 0.041% (colony A: $t = -2.59$; $P = 0.1221$; colony B: $t = -0.20$; $P = 0.8591$) or 0.164% (colony A: $t = -0.08$; $P = 0.9432$; colony B: $t = -1.34$; $P = 0.3118$) (Table 2). Mean survival of termites in the 2-phenoxyethanol treated side was not significantly different from the control in either colony A ($F = 3.07$; df = 2, 6; $P = 0.1207$) or colony B ($F = 2.46$; df = 2, 6; $P = 0.1663$) (Fig. 2).

Search tunnel formation in response to 2-phenoxyethanol residues at two weeks. After 2 wks, residues of 0.082% 2-phenoxyethanol significantly increased the total network length by *C. formosanus* compared with the control (colony C: $t = -3.58$; $P = 0.0372$; colony D: $t = -4.98$; $P = 0.0380$) (Table 3). On day 1, day 2, and day 3, tunnel length was higher in the 2-phenoxyethanol treated side than that in the control

Table 1. Mean (±SE) search length of tunnels by *C. formosanus* in the sand treated with 2-phenoxyethanol (or control) for five days

Colony	Concentration (%, w/w)	Treatment	Length of tunnels (cm)				
			Day 1	Day 2	Day 3	Day 4	Day 5
A	0.041*	2-phenoxyethanol	36.5 ± 16.0	19.9 ± 3.9	6.8 ± 4.2	25.3 ± 5.2	12.2 ± 3.1
		Control	15.2 ± 5.5	7.6 ± 4.9	0.0 ± 0.0	4.8 ± 2.6	7.7 ± 5.8
	0.082*	2-phenoxyethanol	35.2 ± 5.3	40.7 ± 5.9	2.8 ± 2.8	35.3 ± 5.1	7.3 ± 4.3
		Control	9.5 ± 3.4	4.7 ± 1.6	0.7 ± 0.7	4.3 ± 4.3	30.5 ± 0.9
	0.164	2-phenoxyethanol	10.8 ± 1.7	26.8 ± 10.6	2.3 ± 2.3	29.2 ± 11.5	14.0 ± 9.1
		Control	18.0 ± 4.5	39.3 ± 6.4	4.2 ± 4.2	5.8 ± 3.0	14.3 ± 5.4
B	0.041	2-phenoxyethanol	6.0 ± 6.0	59.2 ± 26.2	32.8 ± 14.1	3.7 ± 2.0	0.7 ± 0.7
		Control	1.8 ± 1.8	25.5 ± 11.2	15.8 ± 7.2	7.3 ± 4.2	39.3 ± 5.3
	0.082*	2-phenoxyethanol	11.5 ± 0.5	19.3 ± 11.5	22.0 ± 4.1	20.5 ± 6.5	1.7 ± 0.9
		Control	4.2 ± 4.2	22.0 ± 12.6	12.2 ± 1.0	1.0 ± 1.0	4.3 ± 2.2
	0.164	2-phenoxyethanol	10.8 ± 1.7	26.8 ± 10.6	2.3 ± 2.3	29.2 ± 11.5	14.0 ± 9.1
		Control	4.5 ± 4.5	9.5 ± 7.6	7.8 ± 7.6	0.0 ± 0.0	6.3 ± 6.3

* Significant difference between treated and control values over the whole tested duration at $P = 0.05$.

Table 2. Total (±SE) search length of tunnels by *C. formosanus* in the sand treated with 2-phenoxyethanol (or control) for five days

Colony	Treatment	Length of tunnels (cm) at different concentrations (%, wt/wt)		
		0.041	0.082	0.164
A	2-phenoxyethanol	100.7 ± 20.4	121.3 ± 2.4*	83.2 ± 9.1
	Control	35.3 ± 6.6	49.0 ± 6.9	81.7 ± 9.5
B	2-phenoxyethanol	102.3 ± 47.4	75.0 ± 22.4*	57.7 ± 28.9
	Control	89.8 ± 15.9	43.7 ± 17.9	28.2 ± 19.8

* Significant difference between treated and control values at $P = 0.05$.

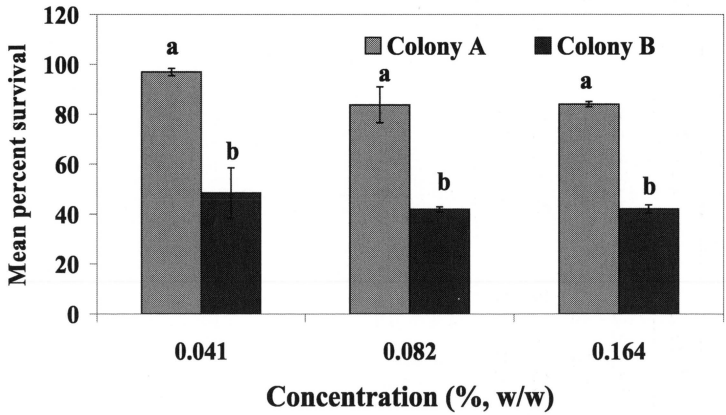

Fig. 2. Mean (±SE) percent survival of termites at different concentrations of 2-phenoxyethanol. Means followed by the same letter within the same colony are not significant different (p > 0.05, Tukey's studentized range test).

side in both colony C (F = 8.88; df = 1, 6; $P = 0.0246$) and colony D (F = 11.59; df = 1, 4; $P = 0.0272$) (Table 3).

Discussion

Delaplane and La Fage (1989) and Campora and Grace (2001) noted that subterranean termites increase tunneling activity near foraging sites. Results presented herein demonstrate that tunneling activity can be enhanced even without a food source nearby with the addition of 2-phenoxyethanol. Increased search activity close to nearby food sources reduces the time for termites to colonize and use and recycle the available nutrients. It is likely that termites use chemosensory sensillae to locate chemical gradients created by cellulose degradation.

Cornelius et al. (2002) reported that methanol extracts of fungus-infested sawdust increased the tunneling activity of *C. formosanus* in sand and argued that these chemicals could be used to improve the efficacy of baits in the field. Our results

Table 3. Mean and total (±SE) search length of tunnels by *C. formosanus* in the sand treated with 2-phenoxyethanol (or control) for five days

Colony	Treatment	Length of tunnels (cm)					Total
		Day 1	Day 2	Day 3	Day 4	Day 5	
C	2-phenoxyethanol	13.5 ± 2.8	13.2 ± 3.9	10.3 ± 3.9	6.0 ± 0.9	5.4 ± 5.4	48.4 ± 7.6*
	Control	4.8 ± 3.0	7.6 ± 3.5	5.5 ± 3.3	2.5 ± 2.5	3.5 ± 3.4	23.9 ± 3.1
D	2-phenoxyethanol	29.2 ± 8.9	19.7 ± 0.2	26.8 ± 7.1	17.2 ± 3.8	9.0 ± 4.0	101.8 ± 8.1*
	Control	18.5 ± 6.2	3.3 ± 3.3	8.3 ± 4.5	3.5 ± 3.5	3.5 ± 3.5	37.2 ± 17.2

* Significant difference between treated and control values at $P = 0.05$.

indicated that application of 2-phenoxyethanol also may increase the likelihood for termites to encounter a food source and might be a useful additive into a liquid insecticide. For example, adding 2-phenoxyethanol to a nonrepellent termiticide may increase the number of termites moving through the toxicant and possibly increase toxicant transfer loads.

Chen et al. (1998) suggested that effort might be necessary to stabilize 2-phenoxyethanol long enough to orient foraging termites in any practical applications. Rapid degradation and volatility of many trail-following substances and pheromones might be overcome by adding stable analogues or chemical protectants (Grace 1990), but this has not yet been demonstrated (Chen et al. 1998). However, 2-phenoxyethanol appears to be very stable in the presence of acids and alkalis, and its loss due to evaporation is mainly affected by temperature (LaPorte et al. 2004). According to Gaudreau and Brazeau (2002), the rate of evaporation of 2-phenoxyethanol in ink stabilizes after 6-8 months. The exact rate of evaporation of 2-phenoxyethanol may also depend on the materials to which it is applied. A recent study indicated that residues of 2-phenoxyethanol on pretreated filter paper remained effective in orienting *C. formosanus* workers up to 13 wks (Ibrahim et al. 2005).

A laboratory assay of trail-following on concentration gradients of 2-phenoxyethanol indicates that *C. formosanus* is able to rapidly detect and respond to gradient changes on 2-phenoxyethanol trails (Fei et al. 2005). The highest concentration of 2-phenoxyethanol in this study caused no significant difference in search tunnel formation between the treated side and the control in these laboratory bioassays. This may have been caused by the behavioral response threshold of termites to this chemical. Our study suggests a potential application of 0.082% 2-phenoxyethanol in the field, although studies are further necessary to determine the best rate or concentration of 2-phenoxyethanol added directly to the nonrepellent termiticides or a baiting system.

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