2-Phenoxyethanol is a Double-Edged Sword against the Formosan Subterranean Termite (Isoptera: Rhinotermitidae)

SANAA A. IBRAHIM[1, 2], GREGG HENDERSON[2*], HUIXIN FEI[2] AND ROGER A. LAINE[2, 3]

[1]Department of Plant Protection, Faculty of Agriculture, Minia University, Minia, Egypt; [2]Department of Entomology, Louisiana State University Agricultural Center, Baton Rouge, LA 70803, USA; [3]Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803, USA

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ABSTRACT In laboratory tests, 2-phenoxyethanol was evaluated for its attraction, toxicity and feeding effects on the Formosan subterranean termite. In choice tests with untreated and 2-phenoxyethanol treated filter paper at 0.012 to 1.92 per cent (wt/wt), it was an attractant but not toxicant at 0.12 and 0.24 per cent, an attractant and toxicant at > 0.48 per cent and the maximum effect was at 0.96 per cent. 2-Phenoxyethanol at 0.96 per cent attracted 90 per cent of the termite workers to the treated filter paper side, killing 85 per cent of the termites within 48 h. In laboratory conditions, residues of 2-phenoxyethanol on pretreated filter paper at 0.96 per cent maintained significant activity in both attraction and toxicity for 11 weeks. Toxicity of 2-phenoxyethanol was via inhalation and contact; dead termites had signs of pink color that first appeared in the head and spread into the legs and abdomen. In choice tests, feeding activity on filter paper treated with 2-phenoxyethanol at 0.12 per cent increased as a function of attraction; whereas this concentration in no-choice tests was not a feeding stimulant. This study points to the value of 2-phenoxyethanol in termite remedial control regimes as a single treatment or as an additive to non-repellent toxic barriers. Attractant, non-toxic and non-feeding deterrent concentrations may allow 2-phenoxyethanol to be a candidate as a bait additive.

KEY WORDS : *Coptotermes formosanus*, 2-phenoxyethanol, attraction, toxicity, feeding effect

INTRODUCTION

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is a major worldwide pest that attacks both living trees and wood constructed buildings. It was first discovered in the USA in 1965 and recently considered to be one of the most destructive insect species in Louisiana (Henderson, 2001). Its damage in South Louisiana expanded to include buildings of historic significance in New Orleans' French Quarter. Unlike other subterranean species, Formosan subterranean termites can establish an aerial colony that can disconnect from the original ground contact. Once established in an area they slowly spread and increase in numbers. Recommended insecticide barriers have encountered many problems in controlling this species (Mauldin *et al*., 1987; Tamashiro *et al*., 1990). Moreover, some insecticides used in controlling termites cause indoor air pollution (Katsura *et al*., 1996), human (Smith *et al*., 2002) and animal health risks (http://www.fluoridealert.org/pesticides/Fipronil.article.Pest.News.htm).

One of the most recent advances in termite control is the development of bait systems (Myles, 1996).

* Corresponding author: E-mail: grhenderson@agctr.lsu.edu

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Toxic baits are locally applied in small amounts, which decrease environmental hazard. Baits in the insecticide markets are mostly slow-acting toxicants, allowing foraging termites to transfer the toxicant to other colony members before functional effects take place (Myles, 1996). However, one concern with termite baits is that they are often bypassed and left uneaten because of competing food sources. Subterranean termites accidentally find baits placed in the ground while they are foraging. Among factors affecting foraging behavior of subterranean termites are volatiles associated with wood and fungi (Grace, 1990; Rust *et al*., 1996).

Bait and barrier additives in the form of attractants, feeding stimulants and trail-following substances are being investigated as a way to direct termites to search where baits or non-repellent toxic barriers are located. Formosan subterranean termites have exhibited trail-following activity in response to pheromone (Tokoro *et al*., 1989) and non-pheromone chemicals (Becker and Mannesmann, 1968; Stowell, 1997; Chen *et al*., 1998). Effective non-pheromone chemicals that act as non-specific trail-following substances may be of great value as additive candidates for toxic baits. In addition liquid attractants may increase termite foraging activity near the non-repellent toxic barriers used for termite control.

2-phenoxyethanol was reported to be a termite trail-following substance (Chen *et al*., 1998). It is extremely safe and ecologically sound; the oral-rat and skin-rabbit LD_{50}s were reported to be 1260 and 5000 mg/kg, respectively (http://www.childscreen.org/2PE.htm). It is an effective preservative added to cosmetics and pharmaceuticals such as antibiotic ointments and antiseptic solutions, eardrops, vaccines and skin creams (http://www.lcminc.ca/productlist.pdf, Wenqin, 2000; Rastogi, 2000; Stolen, 2003). The chemical is used for anaesthetizing some species of fish (Mercier *et al*., 2002). It is used as a solvent for inks, resins, and dyes and as a fixative for perfumes (NIOSH, 1991).

To evaluate the value of this chemical as an additive to non-repellent insecticides including toxic baits and liquid barriers, we investigated its effect on termite behavior as a cellulose treatment. Its persistence and its possible toxic effects were also investigated.

MATERIALS AND METHODS

Chemical and Insect

2-phenoxyethanol (99% purity) was purchased from Aldrich Chem. Co. Inc., Milwaukee,WI. Absolute ethanol (Ethyl alcohol USP, absolute-200 proof, Aaper Alcohol and Chemical Co. DSP-KY-417, Shelbyville, KY) was used as a solvent. Formosan subterranean termites were collected from New Orleans, Louisiana in December 2001 (colony PT), August 2002 (colony WB), March 2003 (colony CNNO) and September 2003 (colony BP). Termites from colonies CR1, CR2, CR3 and CR4 were collected from an island in the Calcasieu River, Westlake, LA in August 2002, November 2002, January 2003 and September 2003, respectively.

Attraction and Toxicity in Response to 2-Phenoxyethanol Concentrations

This experiment was designed in choice-treated filter paper test to investigate the effects of 2-phenoxyethanol on the orientation behavior of the Formosan subterranean termite. A stock solution of 2-phenoxyethanol was prepared by dissolving 1.748 g (AI) in 50 ml ethanol (3.5% WT/V). This stock was used to prepare an additional 6 concentrations (1.75, 0.88, 0.44, 0.22, 0.11 and 0.022% WT/V). Forty units of three-chambered transparent plastic containers were used (17.5 X 8.0 X 4.0 cm each box, 8.0 X 5.75 X 4.0 cm each chamber, Pioneer Packaging Co., North Dixon, KY). Small opening (0.5 cm diameter) was made at the bottom of each of the two inner walls, connecting the three chambers. For all containers, each of the two lateral side chambers was provided with a filter paper (Whatman # 2, Whatman International Ltd, Maidstone, England, 5.5 cm diameter, 0.2285 g average weight and 23.77 cm^2 area). For each concentration, five units were used as replicates in which one lateral side was marked “treated” and filter paper in this side was treated with 125µl of 2-phenoxyethanol solution. Filter paper in the opposite side was left untreated. Controls were similarly prepared except that filter paper in the side marked “treated” received 125µl ethanol. Containers were left 4 h uncovered at ambient conditions for solvent evaporation. Then 250µl of double-dis-

tilled water (DDH_2O) was added to each filter paper. The applied concentrations in relation to the average weight of the filter papers were 0.012, 0.06, 0.12, 0.24, 0.48, 0.96 and 1.92 per cent (wt/wt). Fifty workers and 5 soldiers from colony WB were released in the middle chamber. Containers were kept at laboratory conditions covered with their lids and an opaque black sheet to eliminate the effect of light. For each replicate, number of workers in the two lateral side chambers were counted at 2, 4, 6, 12, 24, 36 and 48 h and containers were rotated 90° after each reading. For each concentration, mean percentages of workers in the untreated, treated and middle chambers were calculated from 7 readings of the 5 replicates. Then the distributions of termite workers between the two lateral side chambers were compared using the paired *t*-test (SAS Institute, 1999). The number of dead workers was recorded on day 2, after which containers were provided in the middle chamber with 20g sand (fine blasting sand # 4, Cement Products Inc., Baton Rouge, LA) moistened with 2 ml DDH_2O. By day 8, the number of living termites in each container was recorded. Percentages of termite mortality on day 2 and day 8 were compared among 2-phenoxyethanol concentrations and the control using analysis of variance with the SAS GLM procedure followed by Tukey's Studentized Range (HSD) Test (SAS Institute, 1999).

Persistence of 2-Phenoxyethanol

Residues of 2-phenoxyethanol on pretreated filter paper with 0.96 per cent (wt/wt) were bioassayed at 1, 3, 5, 7, 9, 11, and 13 weeks. To conduct this experiment, a stock solution of 2-phenoxyethanol was prepared by dissolving 0.874 g (AI) in 50 ml of ethanol. One hundred forty filter papers (Whatman # 2) were equally divided into 280 semicircles. Seventy semicircles were treated with 4.375 ml of the prepared stock solution (62.5 µl each, 0.96% wt/wt). Another 70 semicircles were treated with 4.375 ml of the solvent only and the rest were left untreated. Treated filter paper semicircles were dried on a plastic tray covered with aluminium foil for 4 h at ambient conditions after which, each of the three groups was placed in a small glass jar (9 cm diameter by 9.7 cm height), closed with its lid and kept in the darkness at laboratory conditions for up to 13 weeks. For assaying the residual activity of 2-phenoxyethanol at each interval, 20 plastic containers (11.4 cm diameter by 3.7 cm height) were used. The bottom of each container was equally divided into 2 semicircles using a marker. For each of the 10 replicates, one side received a pretreated semicircle with 2-phenoxyethanol and the other side received an untreated semi-circle with a 6 mm distance between them. Control replicates were handled the same way except that one side received a semicircle pretreated with ethanol only. Filter paper semicircles in both sides were wetted with 125µl double distilled water, and then ten workers were released carefully on the borderline between the two sides. Five replicates received termites from colony CR1 and 5 replicates from colony CR2. Containers were covered with their lids and an opaque black sheet. At 1, 2, 3, 4 and 24 h after treatment, the number of workers in the treated side was counted and containers were rotated 90°. For each interval, the mean percentage of termites in each side was calculated from 5 readings of the 10 replicates and compared using the paired *t*-test (SAS Institute, 1999). The number of living termites in each container was recorded after 24 h and the percentage of mortality at each interval was compared among controls and 2-phenoxyethanol treatment using *t*-tests.

Feeding Activity and Survivorship in Choice Tests

Two concentrations of 2-phenoxyethanol, attractant sublethal (0.12%) and attractant lethal (0.6%) were evaluated for their effects on the feeding activity of Formosan subterranean termite workers. Two stock solutions were prepared by dissolving 0.0219 and 0.1095 g (AI) 2-phenoxyethanol in 10 ml ethanol. Eighteen plastic containers were set up as previously described. However, 20 g of wetted sand was placed carefully in the borderline between the 2 filter paper semicircles. For each container, untreated and treated filter paper semicircles were weighed, then placed and moistened as previously described. Six replicates were performed for each of the 3 treatments including controls. The final concentration of 2-phenoxyethanol

on filter paper was adjusted to be 0.12 or 0.60 per cent (wt/wt). One hundred workers and 10 soldiers were released on the sand placed in the borderline between the 2 filter paper semicircles. Containers were covered and incubated at 26.4°C, 59 per cent RH in complete darkness, then observed every two days for the addition of water if needed. On day 8, the number of living termites was counted, then the treated and untreated filter paper semicircles were gently cleaned using deionized water and a small brush. Filter papers were oven dried at 60°C for 3 h, and re-weighed to calculate food consumption. The experiment was repeated four times using termites from different colonies. For each treatment, mean filter paper consumption was compared between the treated and untreated sides using paired *t*-tests (SAS Institute, 1999). For each colony, the mean survivorship and mean consumption on both treated and untreated filter paper semicircles were compared among treatments using analysis of variance as previously described.

Feeding Activity and Survivorship in No-choice Tests

To evaluate whether 2-phenoxyethanol acts as a termite-feeding stimulant, no choice tests were set up using 6 concentrations. The concentrations were prepared by dissolving 0.1750, 0.0875, 0.0437, 0.0219, 0.0109 and 0.0055 g (AI) in 10 ml ethanol. Seventy plastic containers (5.5 cm diameter by 3.7 cm height) were each provided with a filter paper (Whatman # 2, 0.2285 g average weight). Ten containers were used as replicates for each concentration in which filter paper in each container was coated with 125 µl of the stock solution of 2-phenoxyethanol. Tested concentrations in relation to the weight of filter paper were 0.03, 0.06, 0.12, 0.24, 0.48 and 0.96 per cent (wt/wt). Controls were similarly handled except that the filter paper was coated with 125 µl ethanol. Containers were kept uncovered 4 h at ambient conditions for solvent evaporation, and then filter papers were weighed and moistened with 250 µl DDH_2O. Containers were covered with their lids after adding 50 workers and 5 soldiers from colony CR4, then incubated and checked every 2 days for moisture maintenance. On day 8, the number of living workers was counted. Filter papers were cleaned, dried and food consumption was calculated as previously described. For each parameter measured (mortality or food consumption), among concentrations tested and the control, mean percentage of mortality and food consumption were subjected to analysis of variance as previously described.

The experiment was repeated with termites from colony BP**.** Seven concentrations were prepared by dissolving 0.0044, 0.0088, 0.0132, 0.0176, 0.0219, 0.0264 and 0.0352 g 2-phenoxyethanol in 2 ml ethanol. The concentrations on filter papers were 0.12, 0.24, 0.36, 0.48, 0.60, 0.72 and 0.96 per cent (wt/wt). In addition, worker and soldier mortality was recorded on day 3 and day 8. Mortality on day 3 was corrected for control mortality using Abbott's transformation (Abbott, 1925). Then probit analysis results were established (Finney, 1971).

Toxicity via Contact and Inhalation

To study the route of 2-phenoxyethanol penetration, a previous technique developed by Delgarde and Rouland-Lefevre (2002) was used with some modifications. Whatman # 2-filter papers were fitted on the bottom of 8 plastic containers (5.5 cm diameter by 3.7 cm height, 88 cm^3 air). Four containers were marked "treated" and filter paper in each container was treated with 125 µl of 2-phenoxyethanol solution adjusted to the 72 h LC_{90} on workers from colony BP. Filter papers in another 4 containers were left untreated. The 8 containers of the control were handled the same way except that filter paper in each of the 4 containers marked "treated" received 125 µl solvent only. Containers whose papers were treated with either 2-phenoxyethanol solution or solvent were kept 4 h uncovered at ambient conditions for solvent evaporation. Filter papers in all containers were wetted with 250 µl DDH_2O followed by providing 20 workers from colony CR3. For either control or 2-phenoxyethanol treatment, the 4 containers with treated filter papers and the 4 with untreated filter papers were housed together uncovered in a large plastic container (20 cm diameter by 7.8 cm height, 2451 cm^3 air) that was covered with its lid and incubated at 26.4°C, 59 per cent RH in complete

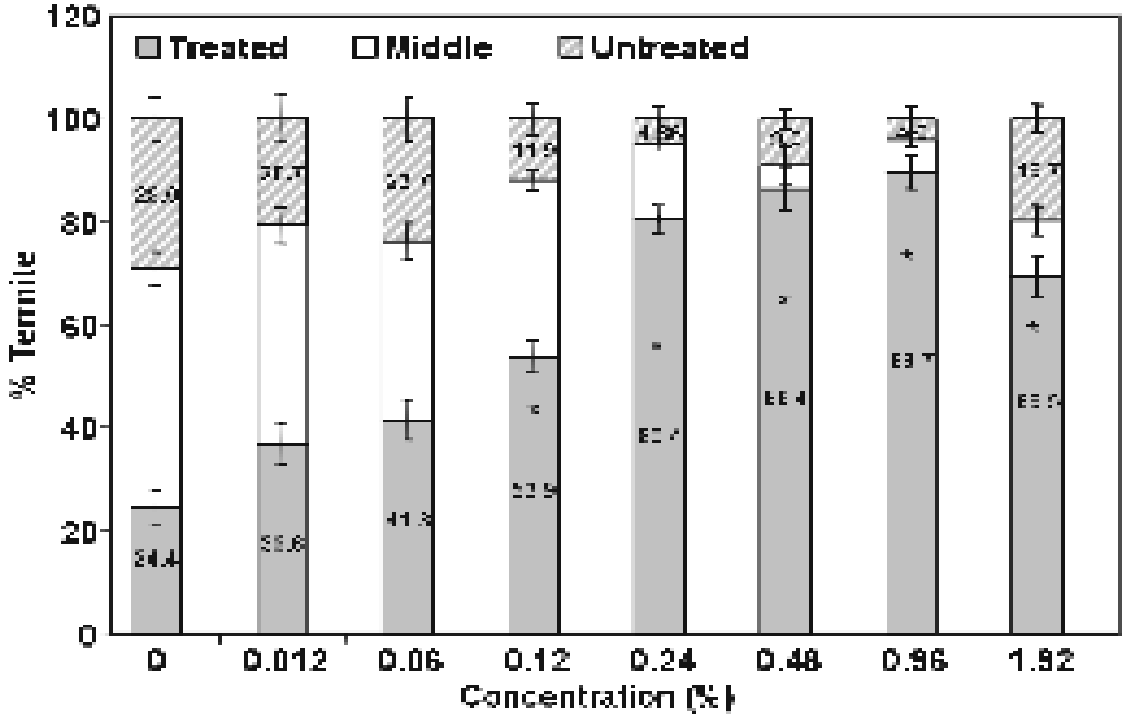

Fig. 1. Mean percentages (± SE) of *C. formosanus* workers (colony WB) in the chambers with untreated and 2-phenoxyethanol treated filter paper and in the middle chamber. Time effect was not significant (F = 1.57, df = 6, 282 and P = 0.1568), therefore data of the 7 readings were pooled as replications. For each concentration, bar graph marked with an asterisk indicates a significant difference in termite distribution between treated and untreated chamber (paired *t*-test).

darkness for 4 days. Treatments were observed daily to provide suitable moisture and recording mortality. The experiment was repeated using termites from colony CNNO. For each colony, mortality via contact and inhalation was compared using analysis of variance as previously described.

RESULTS

Attraction and Toxicity in Response to 2-Phenoxyethanol Concentrations

2-Phenoxyethanol at concentrations that ranging from 0.12 to 1.92 per cent (wt/wt) significantly attracted termite workers to treated filter paper chambers (Fig. 1). Eighty to ninety percent of the termite workers were found in the chamber that had filter paper treated with 2-phenoxyethanol at 0.24, 0.48 and 0.96 per cent (Fig. 1) with only 4 to 9 per cent termite workers recorded in the untreated side (Fig. 1). Results of the paired *t*-tests revealed a significant difference in termite distribution between treated and untreated filter paper chambers in the treatments of 0.12 (t = 3.0772, P = 0.0463), 0.24 (8.3092, 0.0011), 0.48 (7.7310, 0.0015), 0.96 (8.5843, 0.0010) and 1.92 per cent (6.5533, 0.0028). However, the difference was not significant in control treatment (t = -0.4409, P = 0.6821); 0.012 per cent treatment (0.4239, 0.6934) and 0.06 per cent treatment (1.8889, 0.1319; df = 4 for all).

Toxicity of 2-phenoxyethanol was significant at 0.48, 0.96 and 1.92 per cent deposition (Fig. 2). On day 2, mortality was > 85 per cent in 0.96 and 1.92 per cent 2-phenoxyethanol treated filter paper concentrations compared to 1 per cent in the control (F = 58.39; df = 7, 32; P = 0.001, Fig. 2). By day 8, 2-phenoxyethanol at 0.48, 0.96 and 1.92 per cent induced 97 to 100 per cent mortality that was significantly greater when compared to the control or < 0.24 per cent treatments (F = 64.65; df = 7, 32; P = < 0.0001). 2-phenoxyethanol at 0.12 and 0.24 per cent was an attractant but mor-

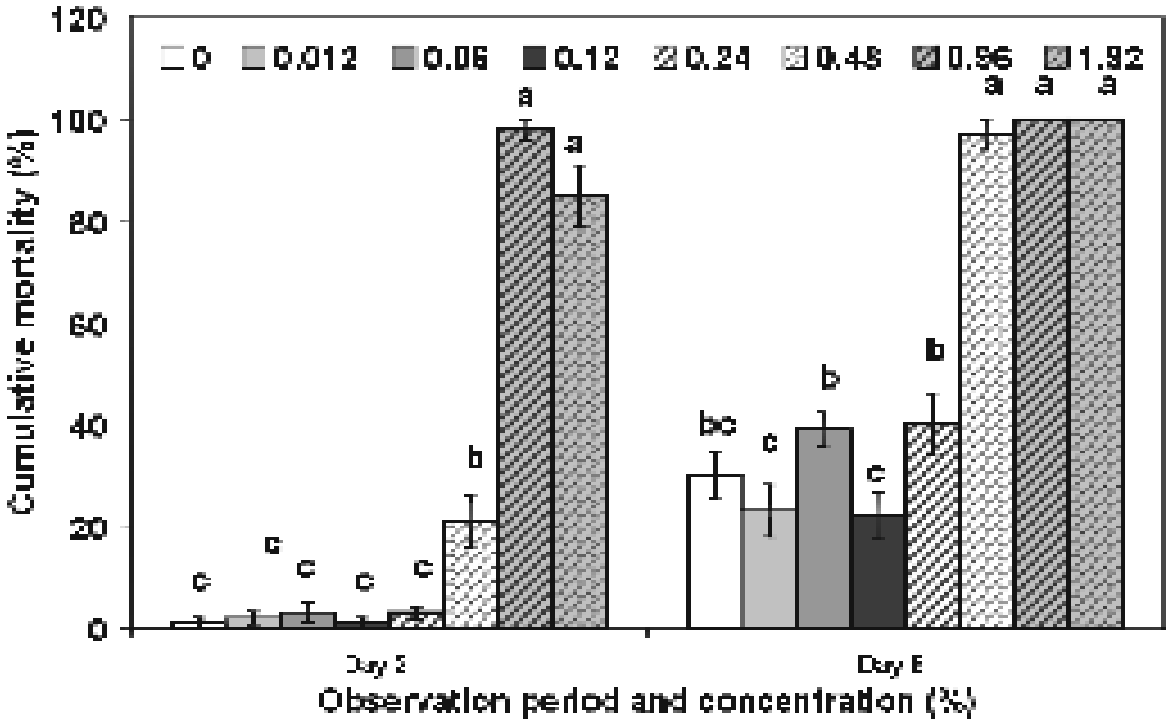

Fig. 2. Mean percentages of cumulative mortality (± SE) on day 2 and day 8 after *C. formosanus* workers (colony WB) were continuously exposed in choice assays to filter paper treated with 2-phenoxyethanol at different concentrations. For each observation period, among concentrations, bar graphs marked by different letters are significantly different (F and P values are 58.39 and 0.001 (day 2) and 64.65 and < 0.0001 (day 8); df values were 7, 32 for all).

tality on day 8 was not significantly different from the controls (Fig. 2).

Persistence of 2-Phenoxyethanol

Residues of 2-phenoxyethanol on pretreated filter paper at 0.96 per cent maintained significant efficiency in attracting 77 to 93 per cent of termite workers tested up to 13 weeks (Fig. 3). Results of paired *t*-test revealed a significant difference in termite distribution between treated and untreated filter paper sides (*t* and *p* values were 3.67, 0.0017 (week 1); 5.19, < 0.0001 (week 3); 4.97, < 0.0001 (week 5); 7.51, < 0.0001 (week 7); 8.54, < 0.0001 (week 9); 11.01, < 0.0001 (week 11); 8.10, < 0.0001 (week 13); df = 9 for all). For the control, 45 to 68 per cent termite workers were in ethanol pretreated filter paper sides were not significantly different from those in the untreated sides (except for a random distribution at 5 weeks interval; t = 1.68, df = 9 and P = 0.0496).

Residues of 2-phenoxyethanol on pretreated filter paper at 0.96 per cent maintained significant toxicity up to 11 weeks. At any of the first 6 intervals, the mortality of termite workers in 2-phenoxyethanol trials was significantly greater than controls (Fig. 3). The *t* and P values were -50.91, < 0.0001 (week 1); -7.95, < 0.0001 (week 3); -6.40, < 0.0001 (week 5); -6.78, < 0.0001(week 7); -5.39, < 0.0001 (week 9); -11.25, < 0.0001(week 11) (df = 18 for all). A dramatic decrease in the toxic effect of 2-phenoxyethanol was observed on week 13 in which mortality was not significantly different from the control (*t* and *p* values were -2.34, 0.056 (week 13). This probably reflects volatile loss of 2-phenoxyethanol to less than lethal levels, but analysis of residual concentration has not been performed.

Feeding Activity and Survivorship in Choice Tests

Consumption of filter paper treated with 2-

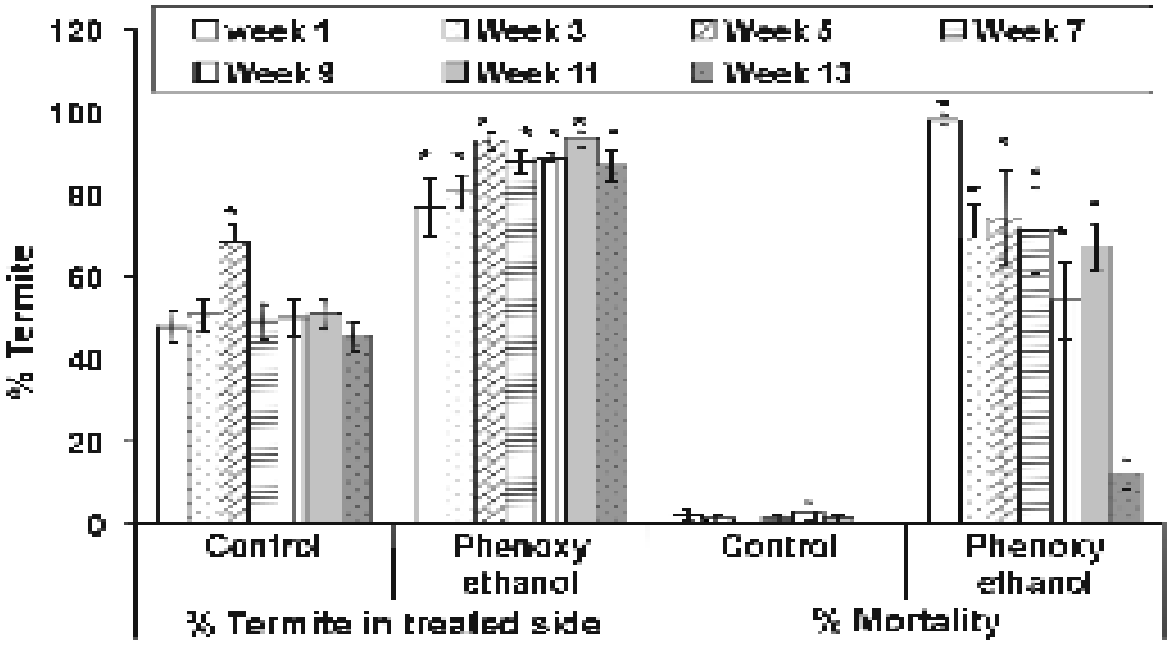

Fig. 3. Mean percentages (± SE) of *C. formosanus* workers (colonies CR1 and CR2) in the treated side, and the 24 h mortality in control and 2-phenoxyethanol treatments. For each treatment, bar graph represents percent termites in treated side marked with an asterisk indicates a significant difference in termite distribution between treated and untreated side (paired *t*-test). For mortality data, a bar graph marked with an asterisk indicates a significant difference between mortality in 2-phenoxyethanol treatment and control (*t*-test).

phenoxyethanol at 0.12 per cent was significantly greater compared to the untreated filter paper (Paired *t*-Test, 4 colonies, Table 1). However, for either control treatment or 2-phenoxyethanol treatments at the lethal level of 0.60 per cent, difference in consumption between treated and untreated filter paper was not significant (Paired *t*-Test, 4 colonies, Table 1). Survivorship and total consumption on both untreated- and 0.12 per cent 2-phenoxyethanol treated-filter papers were not significantly different from the control (ANOVA, 4 colonies, Table 1). Total consumption on both untreated and treated filter paper as well as mean survivorship was significantly less in 0.60 per cent treatment compared to either the control or 0.12 per cent treatment (ANOVA, 3 colonies, Table 1).

Feeding Activity and Survivorship in No-choice Tests

Consumption on < 0.12 per cent 2-phenoxyethanol treated filter paper was not significantly different from the control (colony CR4, Fig. 4). Feeding activity on filter paper treated with > 0.24 per cent was significantly less compared to the control. Moreover, complete inhibition of feeding activity was observed in 0.96 per cent treatment. Mortality was not significantly different from the control in < 0.24 per cent. Percentage of mortality was significantly greater than the control in 0.48 and 0.96 per cent 2-phenoxyethanol treated filter paper treatments (Figure 4).

Data with colony BP confirmed the previous data with colony CR4 in which survivorship and feeding activity in 0.12 per cent 2-phenoxyethanol treatment was not significantly different from the control (Fig. 5). Also, 2-phenoxyethanol treatment at 0.24 per cent caused significantly less feeding activity compared to control and 0.12 per cent, although there was a similarity among the three treatments in survivorship (Fig. 5). As previously found with colony CR4, 2-phenoxyethanol at 0.96 per cent completely inhibited feeding activity and resulted in 100 per cent soldier and worker mortality (Fig. 5). Worker and soldier mortality was significantly

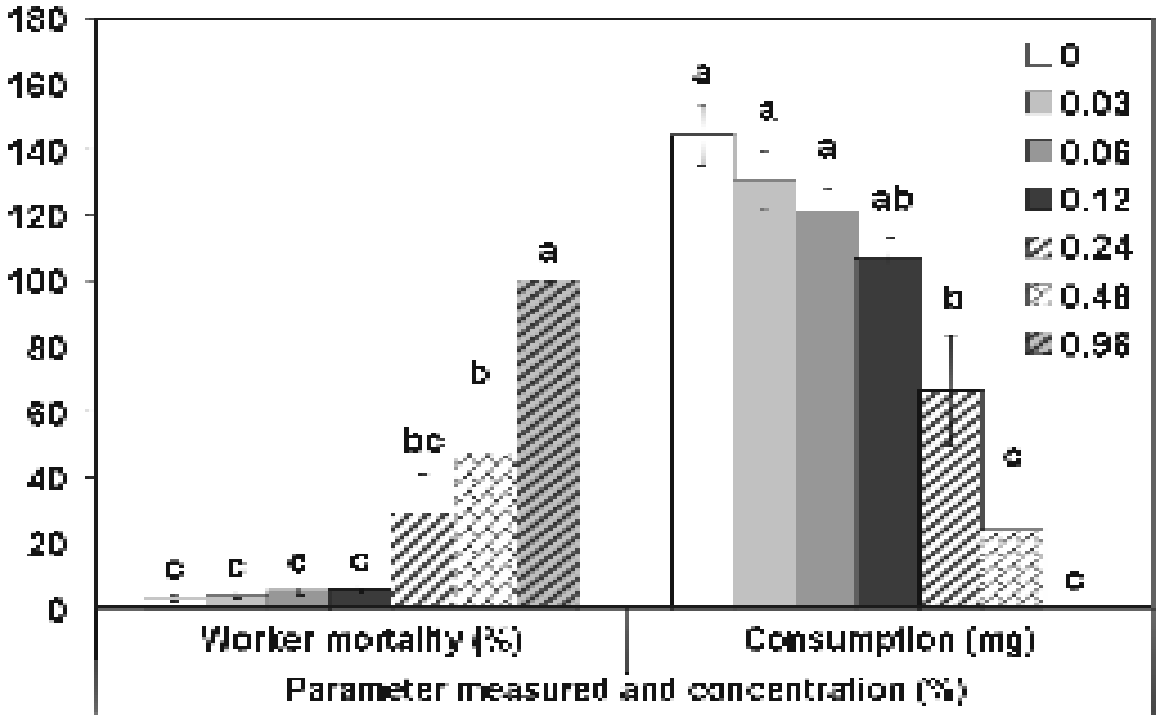

Fig. 4. Mean percentages of mortality (± SE) and mean filter paper consumption as mg (± SE)) when *C. formosanus* workers (colony CR4) were exposed in a no-choice test to filter paper treated with 2-phenoxyethanol for 8 days. For each parameter measured (mortality or food consumption), among concentrations, bar graphs sharing different letters are significantly different (F values are 31.68 and 35.29 for mortality and filter paper consumption, respectively; P and df are < 0.0001 and 6, 63 for all).

Table 1. Filter paper consumption (mg + SE) and survivorship (% + SE) when Formosan subterranean termites were exposed in choice tests to 2-phenoxyethanol (2-PE) treated- and untreated-filter paper for 8 days.

Colony	Treatment (concentration %)	Treated filter paper	Untreated filter paper	*t, df = 4, P*	Both filter papers	Survivorship
WB	Control	17.0 ± 4.05	28.56 ± 11.62	1.1204, 0.3253	45.56 ± 13.99a	57.33 ± 11.64a
	2-PE (0.60)	0.96 ± 0.64	1.0 ± 0.59	-0.0680, 0.9490	1.96 ± 1.09b	5.87 ± 4.94b
	2-PE (0.12)	34.04 ± 4.88	0.0 ± 0.0	0.9980, 0.0022	34.04 ± 4.88a	58.0 ± 6.12a
	F; df = 2, 12; P				27.12 ; < 0.0001	17.03 ; 0.0003
CR1	Control	94.0 ± 16.91	50.0 ± 17.94	1.4538, 0.2197	144.0 ± 17.25	79.4 ± 1.03
	2-PE (0.60)	58.0 ± 22.06	36.0 ± 18.38	0.6421, 0.5558	94.0 ± 21.65	57.6 ± 13.11
	2-PE (0.12)	146.0 ± 19.18	22.0 ± 8.63	6.4816, 0.0029	168.0 ± 22.73	72.0 ± 8.02
	F; df = 2, 12; P				2.18 ; 0.1557	1.53 ; 0.2569
CR2	Control	57.48 ± 6.76	60.48 ± 8.57	-0.4337, 0.6826	116.52 ± 15.26a	85.33 ± 2.86a
	2-PE (0.60)	0.0 ± 0.0	0.0 ± 0.0	—	0.0 ± 0.0b	0.0 ± 0.0b
	2-PE (0.12)	89.92 ± 6.57	4.18 ± 2.34	16.7324, < 0.0001	98.82 ± 7.65a	80.3 ± 8.83a
	F; df = 2, 12; P				1017.81; < 0.0001	87.68 ; < 0.0001
PT	Control	44.06 ± 3.88	42.81 ± 5.75	0.2164, 0.8373	43.43 ± 3.92a	58.99 ± 3.54a
	2-PE (0.60)	12.28 ± 5.27	8.85 ± 4.06	0.4799, 0.6515	10.56 ± 2.96b	15.14 ± 3.62b
	2-PE (0.12)	66.19 ± 5.26	6.35 ± 1.74	11.7972, < 0.0001	36.27 ± 2.94a	58.25 ± 3.93a
	F; df = 2, 12; P				14.21 ; 0.0003	15.26 ; 0.0002

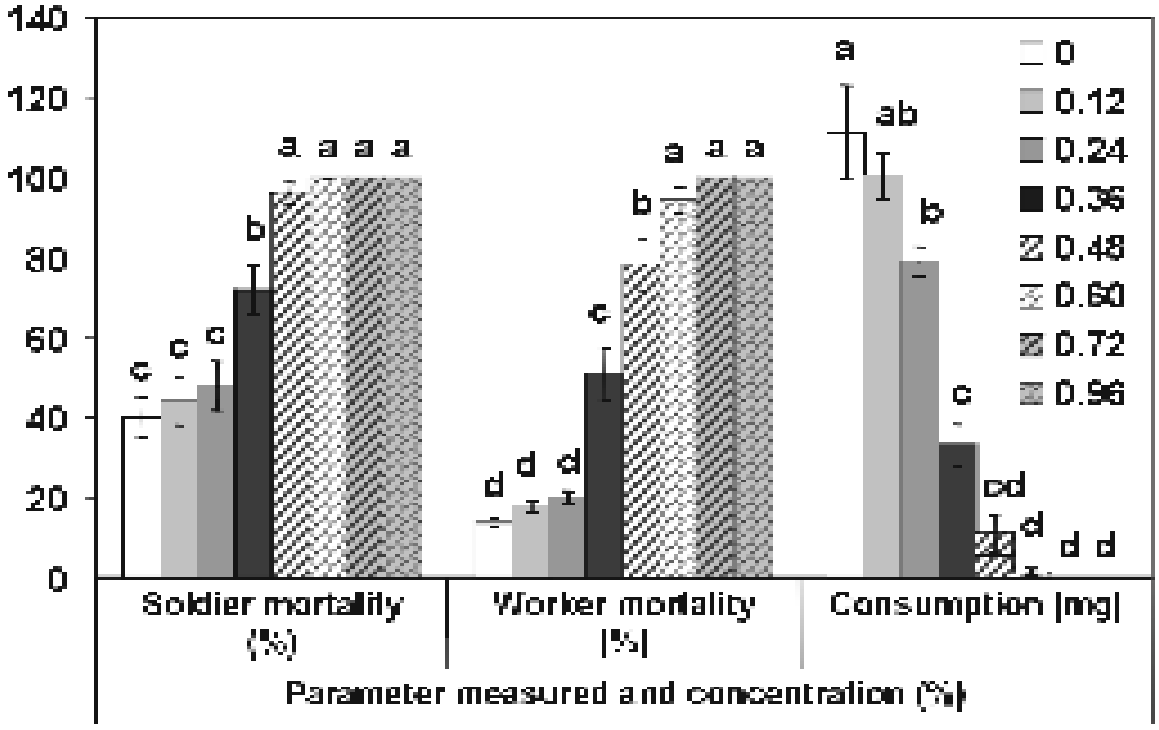

Fig. 5. Mean percentages of mortality (± SE) and mean filter paper consumption as mg (± SE)) when *C. formosanus* workers (colony BP) were exposed in a no-choice test to filter paper treated with 2-phenoxyethanol for 8 days. For each parameter measured (mortality or food consumption), among concentrations, bar graphs sharing different letters are significantly different (F values are 118.34 and 77.37 for mortality and filter paper consumption, respectively; P and df are < 0.0001 and 7, 72 for all).

greater than the control in > 0.36 per cent concentrations. Soldier and workers responded similarly to the contact toxicity of 2-phenoxyethanol. The 72 h LC_{50}s and LC_{90}s were not significantly different between soldiers and workers (based on the overlap of 95% confidence limits, Table 2).

Toxicity via Contact and Inhalation

Workers from colony CNNO responded via contact faster than those from colony CR3; the 72 h LC_{90} established with workers from colony BP showed 100 per cent mortality on workers from colony CNNO after 24 h, suggesting either genetic or phenotypic differences between the colonies. With workers from colony CNNO, inhalation mortality was not significantly different from the control on day 1 and 2. However, mortality via inhalation on day 3 and day 4 was significantly higher compared to the control (Table 3). Termites from colony CR3 responded similarly to

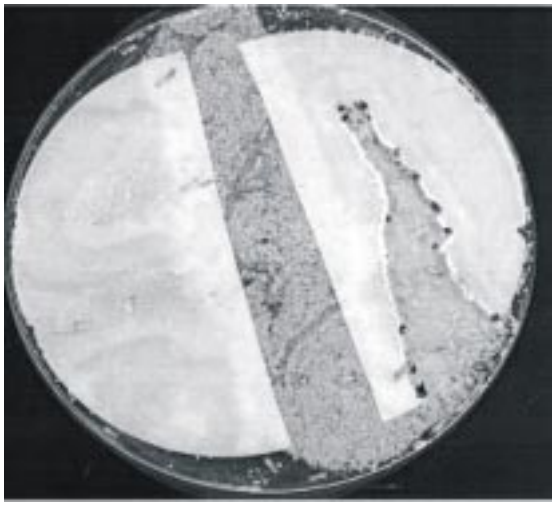

Fig. 6. Image from scanning a replicate in a choice test with untreated and 12 per cent 2-phenoxyethanol treated filter paper after 8 days exposure, showing that most termites (colony CR2) were attracted to treated filter paper and food consumption on the untreated filter paper was negligible.

Table 2. Probit analysis results when *C. formosanus* soldiers and workers (colony BP) were exposed for 3 days in no-choice test to treated filter paper with 2-phenoxyethanol.

Termite	n[a]	Slope ± SE	x^2, *df*, P	LC_{25} (95% CL)[b]	LC_{50} (95% CL)[b]	LC_{90} (95% CL)[b]
Soldier	450	6.27 ± 0.48	25.55, 7, 0.0006[c]	0.42	0.54	0.87
Worker	4500	8.97 ± 1.08	10.43, 7, 0.1655	(0.36-0.47)	(0.46-0.63)	(0.72-1.29)
				0.57	0.68	0.94
				(0.33-0.67)	(0.50-0.77)	(0.82-1.42)

[a]Number of insects in which each probit analysis is based.
[b]Lethal concentrations expressed as g (AI) 2-phenoxyethanol per 100 g filter paper.
[c]χ^2 is significant, indicating non-fitness of probit data on the toxicity line that may due to the few number of soldiers used for each concentration tested.

2-phenoxyethanol by either contact or inhalation. On day 4 all termites were found dead in both routes of exposure compared to < 5 per cent mortality in the controls (Table 3).

DISCUSSION

2-Phenoxyethanol is a potent termite attractant. In choice tests, Formosan subterranean termite workers preferred 2-phenoxyethanol treated filter paper at concentrations ranging from 0.12 to 1.92 per cent (wt/wt). In a previous study, 75 to 80 per cent of Formosan subterranean termite workers and soldiers followed a trail of 2-phenoxyethanol at 0.23µg/cm (Chen *et al*., 1998). A 35 year-old study revealed similar effects with other related glycols on many termite species including Formosan subterranean termites (Becker and Mannesmann, 1968). The mechanism by which 2-phenoxyethanol and the other related (Becker and Mannesmann, 1968) and non-related (2-naphthalene methanol, Stowell, 1997) trail-following substances attract Formosan subterranean termites is unknown. The only similarity between 2-phenoxyethanol and the trail pheromone and non-pheromone substances is that all are primary alcohols sharing only one hydroxyl group (Chen *et al*., 1998). Termites likely have chemical-specific receptors that recognize 2-phenoxyethanol and related compounds. 2-phenoxyethanol is a semi-volatile (Lowe and Southern, 1994) and like other odorants, may be recognized by termites olfactory receptors located in termite antennae (Kaib and Mikus, 1999; Ishida *et al*., 2002).

Fig. 7. Photograph of 3 dead termite workers (colony BP), 2 of them exposed for 24 h to filter paper treated with 2-phenoxyethanol at 1 per cent (WT/WT), showing symptoms of pink color that first appeared in the head and gradually spread to include the legs and abdomen. In this photograph one untreated workers exposed for 24 h to ethanol treated filter paper is included.

There was no evidence that 2-phenoxyethanol acts as a feeding stimulant at concentrations ranging from 0.03 to 0.96 per cent. Moreover, 2-phenoxyethanol at > 0.24 per cent showed a reduction in feeding that was possibly related to mortality. Feeding deterrent effects have been explained as an inhibitory effect on the gustatory receptors located in the termite antennae or in the mouth parts (González-Coloma *et al*., 2002 and http://www.bicco.com/bioneem.html). In choice tests, higher consumption on 0.12 per cent treated filter paper was due to the presence of more termites in the treated side and associated negligible con-

Table 3. Percentages of mortality via contact and via inhalation when C. formosanus workers from colony CR3 and CNNO exposed to filter paper treated with the 72 h LC 90 of 2 phenoxyethanol established with colony BP.

Colony	Route of penetration	Treatment	% Mortality (mean + SE) and days Day 1	Day 2	Day 3	Day 4
CNNO	Contact	Control	0b	2.50 + 1.25c	3.75 + 1.08c	5.0 + 1.77c
		2-PE	100a	100a	100a	100a
	Inhalation	Control	0b	6.25 + 2.07bc	7.50 + 2.16c	8.75 ± 1.08c
		2-PE	0b	12.50 + 5.59b	51.25 + 15.75b	83.75 ± 4.46b
	Treatment effect	F; df = 3,12;	*;	146.06;	34.32;	160.51;
		P	< 0.0001	<0.0001	< 0.0001	< 0.0001
CR3	Contact	Control	0b	1.25 + 1.08b	1.25 ± 1.08b	5.0 ± 1.77b
		2-PE	50.0 ± 1.77a	71.25 ± 4.09a	98.75 ± 1.08a	100a
	Inhalation	Control	0b	0b	1.25 + 1.08b	1.25 ± 1.08b
		2-PE	46.25 + 8.91a	66.25 ± 7.58a	96.25 ± 3.25a	100a
	Treatment effect	F; df = 3,12;	63.45;	73.67;	141.74;	357.19;
		P	< 0.0001	<0.0001	< 0.0001	< 0.0001

* F value is very high

sumption on the untreated filter paper (Fig. 6). At this concentration, 2-phenoxyethanol may be effective as a bait additive. Grace (1990) argued that volatility of bait additive substances that allow for rapid degradation could be solved by selecting stable effective analogues or adding antioxidant adjuvants to stabilize the chemical. Even without additives we believe that 2-phenoxyethanol will have a long shelf life under field conditions, subject to studies of degradation by soil microorganisms. Our study revealed that 2-phenoxyethanol maintained its initial performance in attracting termites for 13 weeks after treatment. Previous studies indicated that 2-phenoxyethanol was stable for 2 weeks at 60°C (Lowe and Southern, 1994, http://ntpdb.niehs.nih.gov/NTP_Reports/NTP_Chem_HS_HTML/NTP_Chem1/Radian122-99-6.html).

Of great interest is 2-phenoxyethanol's toxicity at ≥ 0.36 per cent. At attractant/sub-lethal concentrations, 2-phenoxyethanol may be valuable as an additive to toxic baits and non-repellent toxic barriers that act via contact, expecting to increase the foraging activity and toxicant transfer. In a sand barrier treatments, 2-phenoxyethanol at 100 mg/kg did not significantly affect termite mortality during the first 3 days and termites were actively tunneling in the sand, however by day 11 mortality reached 84.5 per cent and feeding activity was significantly inhibited (Fei *et al*., unpublished data). This suggests that this chemical can be used as both an attractant and toxicant.

There is no information regarding the route of exposure to 2-phenoxyethanol for any insect species. In our study we found that termites were killed by 2-phenoxyethanol via both contact and inhalation. Inhalation and dermal uptake are the most likely routes of human exposure to glycol ethers including 2-phenoxyethanol (Piacitelli *et al*., 1990), however it metabolizes rapidly to phenoxy acetic acid (Roper *et al*., 1997). The mechanism of the toxic action of 2-phenoxyethanol has not been investigated with any insect species; we found that termites exposed in no-choice test to high concentrations of 2-phenoxyethanol (> 0.96%) died within 24 h with a visible sign of a pink color in the head that gradually spread into the legs and abdomen. (Fig. 7). The mode of toxic actions of insecticides, except for chitin metabolic inhibitors,

are similar between insects and humans, however the selectivity may be quantitative. Based on publications available on the toxic effect of 2-phenoxyethanol on mammals, it may affect termites by blocking specific receptors. The toxic effect of 2-phenoxyethanol on mammals was expected to be via blocking NMDA (N-methyl-D-aspartate)-dependent synaptic transmission, leading to several neurological disorders (Stoltenburg-Didinger, 1994; Musshoff *et al*., 1999) and mammalian cytotoxicty (Anselmi *et al*., 2002).

Thus 2-Phenoxyethanol functions in two ways against the Formosan subterranean termite, it attracts termites and kills them at > 0.36 per cent treated filter paper (wt/wt). It may be valuable in a termite remedial control strategy as a single treatment or as an additive to non-repellent toxic barriers. Selecting an attractant, non-toxic and non-feeding deterrent concentration of 2-phenoxyethanol will allow it to be a candidate as a bait additive. The chemical may be of great value as an additive to non-repellent toxic insecticides after the concentration for maximum increased foraging activity is determined. 2-Phenoxyethanol is extremely safe and ecologically sound (NIOSH, 1991; Wenqin, 2000; Rastogi, 2000; Mercier *et al*., 2002; Stolen, 2003). The only reported side effects of 2-phenoxyethanol were a few cases of contact eczema, urticaria and allergy due to 2-phenoxyethanol in aqueous creams, body lotions and vaccines (Lovell *et al*., 1984; Tosti *et al*., 1995; Vogt *et al*., 1998). However, all the aforementioned argue for a low risk of sensitization.

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