# Evaluation of Efficacy and Nonrepellency of Indoxacarb and Fipronil-Treated Soil at Various Concentrations and Thicknesses Against Two Subterranean Termites (Isoptera: Rhinotermitidae)

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ABSTRACT The efficacy and nonrepellency of indoxacarb (150 SC, 150 g [AI]/liter) and fipronil (Termidor SC, 9.1% [AI]) against field-collected eastern subterranean termite, Reticulitermes flavipes (Kollar), and the Formosan subterranean termite, Coptotermes formosanus Shiraki, were evaluated for mortality and penetration into treated soil in laboratory glass tube bioassays. Both insecticides were tested at five concentrations (0, 1, 10, 50, and 100 ppm) and two thicknesses (20 and 50 mm) of treated soil. Indoxacarb caused significantly greater mortality than controls at all treatment thicknesses of  $\geq 10$ ppm, but not at 1 ppm. Concentration and treatment thickness of indoxacarb significantly affected termite mortality. Eastern subterranean termites were significantly more susceptible to indoxacarb than Formosan subterranean termites, but there were no intercolony differences in either species. Termites completely penetrated through all treatment thickness of indoxacarb-treated soil at all concentrations, except one of the six Formosan subterranean termite replicates of 50 mm at 50 ppm, when all termites were killed before tunneling through the treated soil. Fipronil resulted in significantly faster and greater termite mortality than indoxacarb at corresponding concentrations. Concentration and treatment thickness of fipronil also significantly affected termite mortality. There was no intercolony difference in susceptibility to either insecticide in either termite. Both termite species completely penetrated 20-mm treatments of all tested fipronil concentrations, as well as 50-mm soil treated with fipronil at  $\leq 10$  ppm. At 50 and 100 ppm fipronil, termites tunneled only a mean of 87  $\pm$ 0.21 and  $47 \pm 0.18\%$  deep into 50-mm treated soil, respectively, before death. Both insecticides demonstrated a delayed mode of activity and nonrepellency against the two termite species.

**KEY WORDS** *Reticulitermes flavipes, Coptotermes formosanus,* liquid termiticides, penetration, barrier treatment

SUBTERRANEAN TERMITES CAUSE considerable damage to homes and other structures in the United States (Lewis 1997, Su and Scheffrahn 1988). Effective control of subterranean termites has always been an important concern of homeowners and the pest control industry. Unfortunately, termite control seems to be more challenging in the postchlordane era even with the newest chemicals and technologies (Gold et al. 1996, Potter 2000). Nonrepellent termiticides registered in the past 5 yr include fipronil (Termidor), imidacloprid (Premise), and chlorfenapyr (Phantom).

Effectiveness of termiticide-treated soil against subterranean termites depends mainly on the toxicity and the mode of activity of the toxicant, as well as other factors such as termite susceptibility to the insecticide, soil properties (e.g., pH, soil group, particle size, organic matters, and compactness), application protocol, and formulation (Su et al. 1982, Osbrink et al. 2001). Termite penetration of soil treated with termiticides has been considered essential in evaluating termiticide efficacy (Tamashiro et al. 1987, Su et al. 1997). Nonrepellent compounds are preferred for soil treatment because they do not seem to disrupt termite foraging in the treated soil zone and have a delayed mode of action that may contribute to movement of the active ingredient in the colony through trophallaxis and social grooming (Kard 2003).

Because of the changes in subterranean termite control technologies and the needs of the pest control industry, numerous studies have examined the efficacy of insecticides, new formulations, and new active ingredients intended for termite control (Maistrello et al. 2001, Barr and Best 2002, Delgarde and Rouland-Lefevre 2002, Shelton and Grace 2003, Karr et al. 2004). Indoxacarb, an oxadiazine class of novel chemistry, is a newly developed insecticide with high insecticidal activity and low toxicity to nontarget organisms. It acts on sodium channels, neuronal nicotinic acetylcholine receptors, and GABA receptors (Narahashi 2001, DuPont 2003). Indoxacarb was initially registered with Environmental Protection Agency

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(EPA) in 2000 as a "reduced-risk" insecticide for use on vegetable and other crops for controlling of lepidopteran and sucking insect pests (McCann et al. 2001, Tillman et al. 2002). Fipronil, a phenyl pyrazoles class of novel chemistry, was commercialized as insecticide in 1993 and registered for termite market with EPA in 1999 (Aventis 2001). It acts on GABA-receptors and interferes with the passage of chloride ions through chloride channels, thereby disrupting central nervous system activity, resulting in death (Cole et al. 1993). Osbrink et al. (2001) reported Formosan subterranean termite, Coptotermes formosanus Shiraki, susceptibility toward fipronil by exposing termites to treated filter paper. Ibrahim et al. (2003) also tested fipronil toxicity against Formosan subterranean termites by using topical application assays. Termite penetration into fipronil-treated substrates was only examined at concentrations of  $\geq 20$  ppm (Ibrahim et al. 2003), or at 5 ppm (Osbrink and Lax 2002). Neither of the previous studies examined the Eastern subterranean termite, Reticulitermes flavipes (Kollar), nor have they used formulated fipronil. Furthermore, the previous studies used acetone as a solvent.

The objectives of this study were to evaluate the effectiveness and the mode of activity of indoxacarb and fipronil commercial formulations at various treatment concentrations, thicknesses, and time intervals against field-collected eastern and Formosan subterranean termites. We hypothesized that if indoxacarb is similar to fipronil in efficacy and nonrepellency against termites, there would be no difference in termite susceptibility to tested insecticides with various soil thicknesses treated with different concentrations; and eastern subterranean termite is as sensitive as the Formosan subterranean termite to the two tested insecticides.

## Materials and Methods

Target Termites. Three eastern subterranean termite field colonies and two Formosan subterranean termite field colonies collected from Alabama in September 2003 were used in laboratory bioassays. The eastern subterranean termite colonies A2, A15, and A55 were obtained from different locations, at least 1 km apart, in Auburn, Lee County, by using underground open-bottom bucket traps (Hu and Appel 2004). The Formosan subterranean termite colony O3 was collected from Opelika, Lee County, and colony B1 from Fairhope, Baldwin County, by using the same method. Species were identified using a key to soldier caste (Scheffrahn and Su 1994). Termites were maintained in moistened corrugated cardboard rolls in an incubator at 22 ± 1°C, 92 ± 3% RH, and constant darkness and tested within 3 d of collection. Voucher specimens of each colony are preserved in 100% ethyl alcohol and stored in the Insect Collection of the Department of Entomology and Plant Pathology, Auburn University, Auburn, AL.

**Insecticides.** Indoxacarb, (S)-methyl 7-chloro-2,5dihydro2[[(methoxy-carbonyl) [4-(trifluoromethoxy) phenyl]amino]carbonyl]-indeno[1,2-*e*][1,3,4]oxadiazine-4*a*[<sup>3</sup>H]-carboxylate, was provided by DuPont (North Mount Olive, NJ). Formulated indoxacarb 150 SC containing 14.5% active ingredient (wt:wt) was used in this study. Fipronil, 5-amino-1-(2,6dichloro-4-(trifluoromethl) phenyl)-4-((1,R,S)-(trifluoromethyl)sulfinyl)- 1-H-pyrazole-3-carbonitrile, was supplied by BASF Corporation (North Mount Olive, NJ). The formulation used, Termidor SC, contains 9.1% active ingredient (wt:wt). A 1000 ppm stock solution of each insecticide was made by adding 6.667 ml of indoxacarb 150 SC or 10.99 ml of Termidor SC to 1000 ml of distilled water. The stock solutions were serially diluted to desired concentrations. Formulated insecticides instead of technical grade active ingredient were used in this study because they are not only easy to mix and treat soil but also, more importantly, they more closely approximate field applications (Rust and Smith 1993).

Soil Treatment. Soil was collected from the site of the eastern subterranean termite colony A15 (sand, 57.75%; silt, 20.94%; clay, 18.1%; organic matter, 3.2%; pH 7.8). Soil was oven-dried at 60°C for 24 h, sieved (no. 20, 850 µm, Fisherbrand, Fisher, Pittsburgh, PA), and bagged in heavy-duty zip bags at 100 g of sterilized soil per bag. Ten milliliters of a solution containing 0, 10, 100, 500, or 1000 ppm active ingredient was added to a bag to make 0, 1, 10, 50, or 100 ppm (weight active ingredient/weight substrate) soil treatments. Each treatment was replicated four times by serially diluting new stock solutions for each replicate. The bags were sealed and thoroughly kneaded. Treated soil was placed in glass petri dishes (150 by 20 mm) and airdried in a hood for 7 d to allow evaporation of solvents in the formulation. At the time of testing, dry, treated soil was placed into zip bags, again 100 g per bag, and 10 ml of water was added to obtain a 10% (wt:wt) moisture content. Additionally, 500 g of oven-dried and sieved (no. 16, Fisherbrand, Fisher) play sand was wet with 50 ml of distilled water to obtain moist sand.

Test Unit/Bioassay. Modified bioassay units designed by Gahlhoff and Koehler (2001) were used to study treatment efficacy and termite penetration into treated soil (Fig. 1). A moist piece of corrugated cardboard (10 mm by 30 mm) was folded into a z-shape and placed inside at the bottom of a glass test tube (16 mm i.d. by 100 mm high). A 10-mm core of 7% nonnutrient agar (Chem Scientific LLC, Norwood, MA) was plugged into the test tube until it rested on the cardboard. A 20- or 50-mm layer of insecticide-treated soil was centered between two layers of untreated play sand. The total length of the layers of treated soil and untreated sand was 70 mm. A glass funnel was used to introduce the treated soil and sand into test tubes. A cork stopper attached to a needle with wood handle was used to slightly tamp and level each layer. Then, the upper layer of sand was capped with a 10-mm agar plug before a strip of 5 by 30-mm moist filter paper (Whatman no. 1) was folded and placed in the top void of the tube. Fifty workers, at least third instar as determined by body size, and five soldiers of the Formosan subterranean termite or 50 workers and one soldier of the eastern subterranean termite were in-

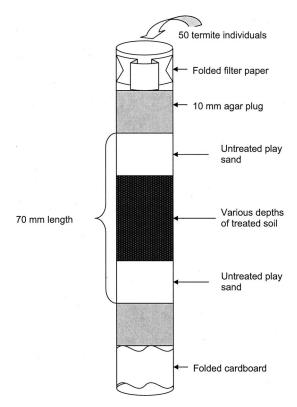


Fig. 1. Bioassay design showing location of termiticidetreated soil centered between two layers of untreated sand and plugged by 7% agar cores.

troduced into the top of each test tube. A piece of plastic wrap (Reynolds Wrap, Richmond VA) was used to cover the top end of each tube to keep unit moist and to prevent termites from escaping.

The responses (mortality and penetration into treated soil) of the two termite species to five concentrations (100, 50, 10, 1, and 0 ppm), each of the two insecticides at two treatment thicknesses (20 or 50 mm), were examined using split-split plot arrangement of treatments. Each treatment was replicated nine times for the eastern subterranean termite (three replicates of three colonies) and six times for the Formosan subterranean termite (three replicates of two colonies) for a total of 150 test units for each insecticide. The test units were held vertically in test tube racks with termites started at the top of the tube. Bioassay units were held in darkness in an incubator at  $22 \pm 1^{\circ}$ C and  $92 \pm 3\%$  RH. Termite mortality and the length of tunneling distance into treated soil were recorded at 3, 7, 14, 21, and 28 d.

Data Analysis. Cumulative mortality and penetration were calculated for each colony and each treatment. Because no significant difference was detected between colonies, pooled means of each treatments with each species or each insecticide from the same time were analyzed using analysis of variance (ANOVA), following the arcsine square-root transformation of proportion of mortality or penetration. Means were separated with Tukey's honestly significant difference (HSD) test (Statistix8 2003). When factor-interaction occurred, a Means Plot was used to analyze how one variable affected the main effect of the other variable, and differences between the two species or the two insecticides were further compared based on treatment thickness or concentration. All data were judged at P = 0.05. Actual percentage of mortality or penetration is reported in the tables and figures.

#### **Results and Discussion**

Efficacy of Indoxacarb against Termites. Significantly greater termite mortality occurred in treatments with  $\geq 10$  ppm indoxacarb-treated soil compared with controls at either treatment thickness (20 or 50 mm) (Table 1; Fig. 2).

Treatment thickness and concentration significantly affected the survival of both termite species (Table 1; Fig. 2). Treatment thickness of 50 mm resulted in significantly greater and more rapid mortality than treatment thickness of 20 mm at any of the concentrations  $\geq 10$  ppm. The 50-mm treatment thickness of 100 ppm resulted in a mortality of  $\approx 100\%$  in both termite species after 3-d exposure, whereas the 20-mm treatment resulted in a similar mortality after 7 d. With decreasing concentration, termite mortality decreased significantly and the time required killing the termite increased significantly. Fourteen days was needed for 50 ppm indoxacarb to kill all the tested termites, whereas at 10 ppm, only 36% (treatment thickness of 20 mm) and 70% (treatment thickness of 50 mm) mortality occurred by the end of the 28-d bioassay. A treatment concentration of 1 ppm was not effective, resulting in <4% mortality during the 28-d trial, regardless of treatment thickness. Analysis of variance of data of 28 d (MS = 17.62, F = 10862, P <0.000) followed by Tukey's HSD comparisons test indicated that the termite mortality at 50 and 100 ppm treatment was significantly greater than that at 10 ppm, which was greater than those at 1 ppm and control, which were not significantly different. Examination of the significant interaction between treatment thickness and concentration, by using a Means Plot, showed that although treatment thickness increased termite mortality, increased mortality was generally associated with increased concentration. The fact that the percentage of mortality in either species increased with increasing exposure period in treatments clearly demonstrated the slow-acting character of indoxacarb in this study.

Eastern subterranean termite was significantly more susceptible to indoxacarb than the Formosan subterranean termite when exposed to 50 or 100 ppm throughout the 14 d (Table 2; Fig. 2). However, comparison of the pooled mortality of the eastern subterranean termite with that of the Formosan subterranean termite showed no significant difference at concentration 10 ppm. In all the treatments, no significant intercolony difference was detected in eastern subterranean termite. In the Formosan subterra-

Component	Source	10		Exposure days				
		df		3	7	14	21	28
R. flavipes <sup>a</sup>	Insecticide	1, 160	MS	7.77	9.927	10.898	12.312	11.487
			F	749.19	694.97	1,616.71	2,863.26	10,100.3
			Р	0.000	0.000	0.000	0.000	0.000
	Concentration	4, 160	MS	16.47	16.66	16.200	15.307	15.124
			F	1,577.13	1,166.24	2,403.07	3,547.26	13,298.6
			Р	0.000	0.000	0.000	0.000	0.000
	Thickness	1,160	MS	0.696	0.489	0.280	0.076	0.084
			F	67.12	34.22	41.18	17.61	76.60
			Р	0.000	0.000	0.000	0.000	0.000
C. formosanus <sup>b</sup>	Insecticide	1,100	MS	12.516	8.238	8.833	8.826	7.725
			F	2,527.07	1,309.50	1,071.87	4,221.49	7,320.29
			Р	0.000	0.000	0.000	0.000	0.000
	Concentration	4, 100	MS	7.067	10.009	10101	10.058	10.0410
			F	1,426.87	1,469.66	1,226.85	4,810.89	9,514.78
			Р	0.000	0.000	0.000	0.000	0.000
	Thickness	1,100	MS	1.557	0.1265	0.064	0.028	0.051
			F	314.32	18.57	7.77	13.13	47.97
			Р	0.000	0.000	0.006	0.001	0.000

Table 1. Effects of insecticide (indoxacarb versus fipronil), concentration, and treatment thickness on cumulative mortality in termites over time

P < 0.05; ANOVA.

<sup>a</sup> Fifty workers (third instar or older) and one soldier per replicate with three replicates.

<sup>b</sup> Fifty workers (third instar or older) and five soldiers per replicate with three replicates.

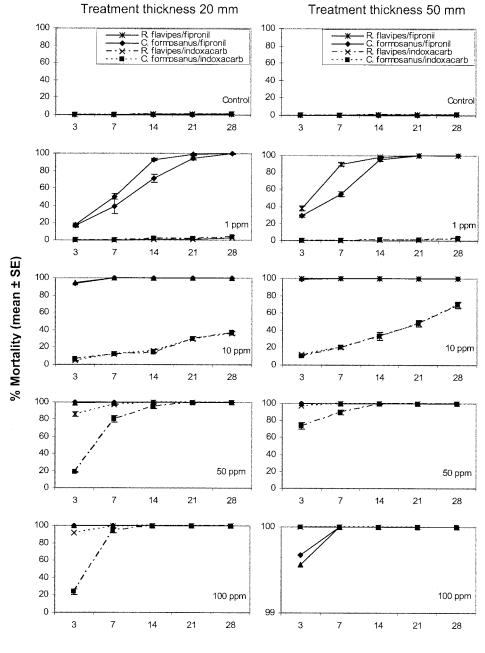
nean termite, the only significant intercolony difference was found at 3 d when colony O3 had twice the mortality of colony B1 in the 100 ppm 20-mm treatment. Yet, the two colonies showed no significant difference in susceptibility in other treatments or at other observation times.

Penetration of Termites into Indoxacarb-Treated Soil (Fig. 3). Both treatment thicknesses of indoxacarb were completely penetrated by eastern subterranean termite in 3 d, regardless of the rate of survival. Similarly, the Formosan subterranean termite tunneled through all indoxacarb treated soil in 3 d except for one replicate of the 50-mm thickness of 50 ppm, in which they tunneled 40 mm into treated soil before death. There was no clear difference in tunneling activity between the two termite species ( $F_{1,143} = 1.00$ ; P = 0.32), the two treatment thicknesses ( $F_{1,143} = 1.51$ ; P = 0.22), or the five concentrations ( $F_{4,143} = 1.00$ ; P =0.41).

Efficacy of Fipronil against Termites. Concentration significantly affected the mortality of the eastern subterranean termite and the Formosan subterranean termite. Both species suffered  $\approx 100\%$  at treatment concentrations  $\geq 10$  ppm by 7 d and at the lowest concentration of 1 ppm by the end of the 28-d bioassay, regardless of treatment thicknesses (Table 1; Fig. Although treatment thickness might have affected termite survival, the nearly 100% mortality concealed differences statistically among fipronil treatments of  $\geq$ 10 ppm. Nevertheless, significantly mortality in the 1 ppm bioassay was observed in 50-mm treatment than that of 20 mm starting from 3 d to 21 d. If the observation period in this study was <3 d, differences between concentrations or between treatment thicknesses at concentrations  $\geq 10$  ppm fipronil might have been detected.

The relative toxicity of technical grade fipronil to rhinotermitid species has been evaluated by several studies. Ibrahim et al. (2003) reported an LD<sub>50</sub> < 2.0 ng per termite after 72-h treatment of the Formosan subterranean termite. They also reported 100% mortality of termites at 48 h after exposure to 50 g sand treated with 10 ml of fipronil at rates of 0.01–0.125% in a choice assay. Osbrink et al. (2001) reported that the LT<sub>50</sub> and LT<sub>90</sub> values of the Formosan subterranean termite workers fed on fipronil-treated filter paper (630.65  $\mu$ g/cm<sup>2</sup>) were 164–249 and 240–330 min, respectively. Osbrink and Lax (2002) observed 100% mortality of the Formosan subterranean termite exposed to sand treated with 5 ppm fipronil within 10–20 d after treatment.

This study did not detect significant interspecies or intercolony differences in susceptibility to fipronil at concentrations  $\geq 10$  ppm of either treatment thickness, perhaps due to the similar mortality at the first designated recording date (Table 2). However, further analysis revealed inconsistent sensitivity of both species at 1 ppm fipronil. At fipronil 1 ppm, the 20-mm treatment thickness resulted in greater mortality in the Formosan subterranean termite than the eastern subterranean termite throughout the 28 d bioassay, although statistically significant difference was detected only at 14 d ( $F_{1,13} = 2.83$ ; P = 0.015). The opposite was observed in treatment with 50-mm treated soil: eastern subterranean termite displayed greater mortality than the Formosan subterranean termite before both reached 100% mortality, and a statistical difference was detected at 3 d ( $F_{1,13} = 5.74$ ; P =0.03) and 7 d ( $F_{1,13} = 45.3$ ; P = 0.00). Because there was no intercolony difference, the possible explanation to this inconsistency could be intracolony difference in vigor or other factors. Osbrink et al. (2001) reported intracolony difference and explained that a portion of the colony might possess mechanisms that enhanced detoxification or limited termiticide uptake. Forschler (1994) indicated that natural vigor and size



Exposure days

Fig. 2. Cumulative percentage of mortality of the eastern subterranean termite and the Formosan subterranean termite exposed to indoxacarb or fipronil at various thicknesses and various concentrations.

of individual termites also might account for difference in bioassay results. Osbrink et al. (2001) and Shelton and Grace (2003) found no correlation of the Formosan subterranean termite worker body size with susceptibility to fipronil. In this study, the body weights for both tested termites were not measured.

Su and Scheffrahn (1990) found that the Formosan subterranean termite was consistently less susceptible

to topically applied insecticides than eastern subterranean termite. Beal and Smith (1971) reported that it took longer to kill the Formosan subterranean termite in soil plate tests with chlordane than it did to kill *Reticulitermes* spp. Osbrink et al. (1987) showed the Formosan subterranean termite to be less susceptible to the fumigant sulfuryl fluoride than the other rhinotermitids tested. Osbrink et al. (2001) found that

Table 2. Effects of species (*R. flavipes* versus *C. formosanus*), concentration, treatment thickness, and the interactive effect of concentration and treatment thickness on cumulative termite mortality over time in indoxacarb (150 SC) and fipronil (Termidor SC) bioassays

Component	Source	df			Exposure days				
				3	7	14	21	28	
Indoxacarb	S		MS	2.067	0.36	0.024	0.001	0.000	
			F	239.64	43.19	5.74	0.31	0.05	
			Р	0.000	0.000	0.018	0.578	0.822	
	С	4,130	MS	8.42	15.156	18.501	18.283	17.619	
			F	976.63	1,808.21	4,423.79	10.558.2	10,862.5	
			Р	0.000	0.000	0.000	0.000	0.000	
	Т	1,130	MS	2.670	0.311	0.111	0.069	0.231	
			F	309.63	37.12	26.43	39.56	142.23	
			Р	0.000	0.000	0.000	0.000	0.000	
	C * T	4,130	MS	0.961	0.064	0.053	0.056	0.233	
			F	111.50	7.65	12.57	32.36	143.83	
			Р	0.000	0.000	0.000	0.000	0.000	
Fipronil	S	1,130	MS	0.003	0.076	0.022	0.009	0.001	
			F	0.38	5.27	2.09	1.66	0.84	
			Р	0.537	0.023	0.150	0.200	0.361	
	С	4,130	MS	16.419	14.707	13.25	13.80	13.991	
			F	2,064.39	1,018.58	1,267.50	2,660.49	23,734.4	
			Р	0.000	0.000	0.000	0.000	0.000	
	Т	1,130	MS	0.230	0.209	0.174	0.028	0.001	
			F	28.87	14.48	8.69	5.33	1.05	
			Р	0.022	0.050	0.090	0.230	0.307	
	C * T	4,130	MS	0.105	0.190	0.175	0.027	0.000	
			F	13.23	13.14	16.75	5.12	0.67	
			Р	0.000	0.000	0.000	0.001	0.648	

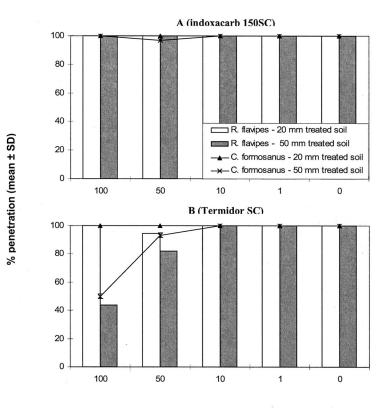
For *R. flavipes*, 50 workers (third instar or older) and one soldiers per replicate with three replicates. For *C. formosanus*, 50 workers (third instar or older) and five soldiers per replicate with three replicates. P < 0.05; ANOVA. C, concentration; S, species; T, thickness.

when being tested on the surface of fipronil-treated filter paper, the Formosan subterranean termite demonstrated greater sensitivity than termite *Reticulitermes virginicus* (Banks), but the opposite was true when 1% Three-In-One Household Oil (Boyle-Midway, NY, NY) was added to fipronil. They suggested that the addition of oil increased fipronil's speed of intoxication for *R. virginicus* but not the Formosan subterranean termite. In this study, the Formosan subterranean termite was consistently less susceptible to indoxacarb than the eastern subterranean termite.

Termite intercolony difference in susceptibility to insecticides also has been reported (Su and La Fage 1984, Osbrink et al. 2001, Shelton and Grace 2003). Despite that large intercolony differences in response to insecticides is common (Su and La Fage 1984), there were incidences where differences in susceptibility between colonies were not detectable. Osbrink et al. (2001) and Ibrahim et al. (2003) reported similarity among the Formosan subterranean termite colonies in their susceptibility toward fipronil.

Penetration of Termites into Fipronil-Treated Soil. Analysis of pooled data showed that the penetration of to both termite species into fipronil-treated soil (Fig. 3) was significantly affected by treatment thickness  $(F_{1,143} = 38.03; P = 0.00)$  and concentration  $(F_{4,143} =$ 21.37; P = 0.00), but not by termite species  $(F_{1,143} =$ 1.47; P = 0.23). Termites penetrated completely through the 20-mm treated soil at all concentrations, except for one replicate of nine with eastern subterranean termite at 50 ppm, in which eastern subterranean termite tunneled <10 mm before death. However, termites only made a complete penetration of 50-mm thickness at concentrations  $\leq 10$  ppm. As the concentration increased, the percentage of penetration into the 50-mm thickness decreased, and greater inhibition of penetration was noted for 100 ppm (an average of 47% of the 50-mm soil) than 50 ppm (an average of 87% of the 50-mm soil). However, the lack of penetration was not necessarily an indication of repellency for fipronil at greater concentrations, but due to relatively rapid mortality of termites before they were able to tunnel through the treated soil. Concentrations of 50 and 100 ppm resulted in 100% mortality of both termite species within 3 d. A close examination of the distribution of termites inside the glass tube test unites showed the an average of 67 or 33% of termites died in tunnels above or inside treated soil at 100 ppm, and 65 or 35% at 50 ppm, suggesting a lack of repellency for fipronil.

The nonrepellency of technical grade fipronil at low rates has been reported previously (Henderson and Forschler 1997, Ibrahim et al. 2003). Ibrahim et al. (2003) reported that fipronil showed no repellency to the Formosan subterranean termite when exposed to 50 g of sand treated with 10 ml of 0.01 or 0.0625% fipronil solution [equivalents of 20 and 125 ppm (wt: wt)]. Repellency was observed only at the highest concentration of 0.125% fipronil-treated sand [equivalent of 250 ppm (wt:wt) ] because significantly lower number of dead termites was found in the treated chambers compared with untreated chambers. Interestingly, they detected no repellency when termites were exposed to fipronil-impregnated filter papers with the same concentrations. The results of this study verified that fipronil concentrations ≤100 ppm were



Treatment Concentration (ppm)

Fig. 3. Cumulative percentage of penetration (mean  $\pm$  SD) of the eastern subterranean termite and the Formosan subterranean termite into soil treated with indoxacarb (A) or fipronil (B) at various thicknesses and various concentrations by 7 d.

nonrepellent to both eastern subterranean termite and the Formosan subterranean termite. These results contrast with the Osbrink and Lax (2002) study, which found that the Formosan subterranean termite only penetrated <55% of any of the 50-mm substrates (sand, soil, and or clay) treated with 5 ppm fipronil by 30 d in a similar glass tube test. This contrast may reflect variation between colonies/individuals or a difference in experimental design.

Osbrink and Lax (2002) reported that fiproniltreated sand had significantly greater and faster mortality of the Formosan subterranean termite workers, from susceptible or unsusceptible colonies, than treated soil or clay. This profound substrate effect also was observed by other researchers with other insecticides (Smith and Rust 1990, Forschler and Townsend 1996, Gold et al. 1996, Osbrink and Lax 2002). Soil or clay has a high colloidal fraction which greatly increases surface area and chemical binding sites that promotes hydrogen bonding and hydrophobic binding of hydrophobic insecticides (Saltzman and Yaron 1986). Sand retains more of the toxicant on the particle's surface than other substrates (Harris 1972) and may maximize pesticide performance. In this study, soil from a termite habitat was treated for the bioassay because such treatment can provide more accurate insight into effectiveness.

In this study, indoxacarb and fipronil were slowacting and had excellent nonrepellency against the two tested termite species. Fipronil performed significantly better than indoxacarb in efficacy against the tested species at corresponding concentrations, with the exception of 100 ppm for 50 mm when both insecticides resulted in 100% mortality at the first observation day (Table 1; Fig. 2). Eastern subterranean termites were significantly more susceptible to indoxacarb than the Formosan subterranean termites and similar to the Formosan subterranean termites in susceptibility to fipronil. There were no intercolony differences in either species.

New chemical treatment strategies for termite control take advantage of these characteristics and termite social behaviors. Ideally, termites will be contaminated unknowingly by tunneling through/within treated zones or ingesting treated food, continue their normal routine, and be able to transfer the toxicant to other uncontaminated colony mates though social interactions such as grooming, contact, and trophallaxis, before dying (Myles 1996, Kard 2001). Henderson and Forschler (1997) reported that fipronil could be effectively used as baits against the Formosan subterranean termite at levels 100 times lower than hexaflumuron. The effectiveness of fipronil as a liquid termiticide for treating the soil around structures also has been confirmed in several field tests (Potter 2000, Waite and Gold 2004). The theorized horizontal transfer of technical grade fipronil from exposed to unexposed the Formosan subterranean termite has been confirmed by Ibrahim et al. (2003) and formulated Termidor SC by Shelton and Grace (2003). Future investigations should be conducted on potential transfer of indoxacarb within and between workers and soldiers, and the potential transfer of both insecticides from foragers to the young or reproductives, the threshold dose/concentrations, and the threshold treated-untreated ratios in laboratory and field tests.

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