Feeding Inhibition and Mortality in *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) After Exposure to Imidacloprid-Treated Soils

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ABSTRACT Feeding inhibition and mortality of *Reticulitermes flavipes* (Kollar) exposed to sand, sandy loam, loam, and silty clay loam soils treated with several concentrations of imidacloprid were studied using bioassay techniques under laboratory conditions. Termite workers stopped feeding after exposure to treated soils. Differences in feeding reduction varied among the soil types. Based on the magnitude of the *F*-statistics, the effect of imidacloprid on the reduction of termite feeding was greatest in sand followed by sandy loam, loam, and silty clay loam soils. Soil properties such as organic matter content, silt and clay proportions, pH, and cation exchange capacity were suggested to affect the bioavailability of imidacloprid. Similar soil effects on mortality were observed in termites continuously exposed to treated soil for 21 d. In three of four soils tested, susceptibility to imidacloprid was not affected by the source of the termites tested.

KEY WORDS subterranean termite, imidacloprid, termite control, soil type

IMIDACLOPRID IS AN insecticide exhibiting low mammalian toxicity. It acts on the insect nervous system by attaching to the acetylcholine binding sites, called nicotinergic receptors, on receiving nerve cells (Abbink 1992). This mode of action prevents transmission of information at these binding sites, leading to a lasting impairment of the nervous system and eventually death of the insect (Schroeder and Flattum 1984). Mammals have few nicotinergic receptors; thus, imidacloprid has very low mammalian toxicity (Sattelle et al. 1989).

Soil-applied termiticides are meant to kill termites or to prevent them from attacking structures. Kard (1997) reported that current termiticides could prevent termite attack for one to > 15 yr, depending on the insecticide and geographic location in the United States. At some sites noted in the study, termiticides were less effective than at other locations. Failure of termiticides to control termites is often related to factors other than the active ingredient (Su and Scheffrahn 1990).

Soil consists of a heterogeneous mixture of mineral particles and organic matter separated by air and water-filled spaces. The heterogeneity and interactions between these phases (solid, liquid, and gas) makes it difficult to predict the precise influence of a soil type on external additives, such as a termiticide. Soil properties affecting insecticide activity include pH, moisture, temperature, microbial fauna, and organic matter content (Harris 1972, Tashiro and Kuhr 1978, Chapman et al. 1982, Macalady and Wolfe 1983, Felsot 1989). Previous studies have indicated that soil type (Lange and Carlson 1956; Harris 1966, 1972; Campbell et al. 1971, Smith and Rust 1993, Forschler and Townsend 1996) is a major factor influencing insecticide performance. A study by Beal (1980) of similar termiticide test sites has shown that they vary in textural component composition of soils (percentage of sand, silt, and clay). Insecticide interactions with soil are complex and cannot be extrapolated from chemical structure alone (Harris 1972). Our study was conducted to determine the effect on the feeding behavior and mortality of the eastern subterranean termite, *Reticulitermes flavipes* (Kollar), when exposed to imidacloprid-treated soils.

Materials and Methods

Termites. Worker termites from two field colonies (A and B) were used in this study. Colonies were separated by ≈1.5 km and located west of the Purdue University campus (West Lafayette, IN). Soldier termites from both colonies were collected and identified as *R. flavipes* (N. Hostettler, personal communication). Termites were trapped and handled according to the method of Ramakrishnan et al. (1999), and at the time of assay had been held in the laboratory ≤ 14 d.

Soils. Soil types used in this study were sand, sandy loam, loam, and silty clay loam. Sand (natural grain silica) was purchased (USA Sands of Time, Ottawa, IL). Sandy loam was collected (top 5–10 cm) from a 0.5-ha apple orchard on the Bayer Ag Corporation's

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research farm (Howe, IN). Loam and silty clay loam soils were collected from the top 5–10 cm from open pits (30–60 cm in depth) at the Purdue Agronomy Research Center (West Lafayette, IN). After collection, soils were analyzed (Soil Survey Staff 1999) for structural composition (i.e., percentage of sand, silt, and clay), pH, cation exchange capacity and percentage of organic matter at Midwest Laboratories, Inc. (Omaha, NE).

Field collected soils were returned to the laboratory and dried the same day in either of two ways depending on desired use. Soils used in the feeding study were spread on a tray and allowed to dry at room temperature for 24–48 h, then treated with imidacloprid, and then immediately used in assays. Soils used in the mortality study were spread on a tray and placed in an oven at 60°C for 24 h, then treated, and then immediately used in assays.

Soil Treatment. Premise 75 WP (imidacloprid active ingredient) was obtained from Bayer Ag Corporation (Kansas City, MO) and serially diluted to provide a range of concentrations (0, 0.5, 1, 5, 10, 20, and 50 ppm) used in the feeding study. In the mortality study the concentrations used were 0, 0.1, 0.5, 1, 5, and 10 ppm in sand and 0, 2.5, 5, 10, 15, 25, 35, and 50 ppm in sandy loam, loam, and silty clay loam soils.

One hundred grams of either oven or air dried soil was placed into 3.7-liter plastic bags. Each termiticide solution was added to obtain field moisture levels: 10% in sand, 15% in sandy loam, and 20% in loam and silty clay loam. This was determined by weight of active ingredient per weight of air/oven dried soil. Untreated controls received distilled water only. After pouring the termiticide solution onto the soil, it was thoroughly mixed into the soil by gloved hands to prevent cross-contamination.

Feeding Study. Termites from colony A were used in the feeding study. One soil type was assayed per week during June 1997. For each soil type, new serial dilutions of imidacloprid were prepared and used to treat the soil. Treated soil was divided evenly into three glass petri dishes (100 by 15 mm) so that each dish was approximately two-thirds full. There were three replicates per concentration for each soil type. Approximately 100 adult workers (measured gravimetrically) were placed into each petri dish. Dishes were sealed with parafilm, wrapped in aluminum foil, placed in plastic storage boxes (4.2 liters) which were then placed in a benchtop environmental chamber set at 26°C. After 24 h the termites were provided an oven-dried (60°C for 24 h), preweighed piece (2.0 by 2.5 cm) of Whatman No. 1 filter paper moistened with water. Filter paper was used because it does not contain glue often found in cardboard food sources. The dishes were resealed with parafilm and stored again. Termites were allowed to feed for 24 h, then the filter paper was removed and cleaned. Fecal matter and debris were removed from the filter paper with a gentle stream of deionized water. The paper was dried at 60°C for 24 h and reweighed.

Mortality Study. Two factors were evaluated in this study: imidacloprid concentration (seven or eight quantitative levels) and termite colony (two qualitative levels [colonies A and B]). The design was a 7 × 2 factorial in sand and an 8 × 2 factorial in sandy loam, loam, and silty clay loam. Each factor level combination was replicated three times in each of four trials (i.e., one trial per day for four consecutive days) during July (sand), August (loam), September (silty clay loam), and October (sandy loam) 1997.

Table 1. Analysis of soils used in feeding inhibition and mortality studies of imidacloprid-exposed eastern subterranean termites

Soil type	Soil composition				pH	Cation exchange capacity (meq/100g)
	% sand	% silt	% clay	% organic matter		
Sand	98	1	1	0.1	6.5	1.4
Sandy loam	72	18	10	1.4	6.3	6.8
Loam	46	32	22	1.0	6.3	10.9
Silty clay loam	18	48	34	0.7	5.2	18.3

For each soil type, enough imidacloprid was serially diluted at the beginning to treat all the soil in all the trials. Between trials, imidacloprid solutions were stored in a refrigerator in parafilm-sealed flasks. Treated soil was divided evenly into three glass petri dishes (60 by 15 mm) so that each dish was approximately two-thirds full. Untreated controls received distilled water only. Twenty-five termite workers and a small piece of moistened cardboard, as a source of food, were placed into each dish. Dishes were handled similar to the feeding study. Three days after the start of the test, the dishes were opened and the number of live termites was counted and recorded; dead termites were removed from the dish and discarded. After counting, the dishes were resealed and returned to the environmental chamber. This procedure was repeated after 7, 10, 14, and 21 d of incubation.

Statistical Methods. SAS procedures were used in all statistical analyses (Schlotzhauer and Littell 1987, SAS Institute 1990). In the feeding study a one-way analysis of variance (ANOVA) was conducted for each soil to determine the effect of imidacloprid on food consumption. Means were separated by Tukey's honestly significant difference (HSD) test.

In the mortality study, percentage of mortality was transformed by arcsine square root as the mortality ranged from 0 to 100% (Agresti 1990). ANOVA of the transformed variable was conducted by day for each soil type. When the two-factor interaction was significant on day 21, the two factors were analyzed like a two-factor experiment. Transformed means were separated by Tukey's HSD test.

Results

Soil pH ranged from 5.2–6.5 for each of the soils tested (Table 1). Differences in structural components included higher percentage of silt in the silty clay loam (48%) compared with the loam (32%) and sandy loam (18%). The trend was similar for the percentages of clay, being highest in the silty clay loam

Table 2. Consumption of Whatman No. 1 filter paper by *R. flavipes* workers following exposure to soils treated with varying concentrations of Premise 75 WP (imidacloprid)

Premise 75 WP concn, ppm	Consumption, mg (mean ± SE)			
	Sand	Sandy loam	Loam	Silty Clay Loam
0	14.4 ± 0.1a	17.4 ± 0.5a	11.0 ± 0.6a	6.5 ± 0.6a
0.5	1.6 ± 0.3b	14.0 ± 0.6b	11.6 ± 0.2b	7.4 ± 1.3a
1	0.9 ± 0.3bc	11.0 ± 1.2b	11.7 ± 0.4b	7.1 ± 0.6a
5	0.9 ± 0.1bc	3.1 ± 0.6c	1.7 ± 1.3c	0.9 ± 0.7b
10	0.4 ± 0.1cd	2.2 ± 0.2c	0.5 ± 0.5c	0.7 ± 0.0b
20	0.4 ± 0.0cd	1.5 ± 0.4c	0.4 ± 0.3c	0.9 ± 0.0b
50	0.0 ± 0.0d	1.4 ± 0.8c	0.0 ± 0.0c	1.0 ± 0.0b
	F = 799.3	F = 93.2	F = 87.1	F = 25.1
	df = 6, 14	df = 6, 14	df = 6, 14	df = 6, 14
	P = 0.0001	P = 0.0001	P = 0.0001	P = 0.0001

Means within a column and followed by the same letter are not significantly different (Tukey HSD test [SAS Institute 1990]). Each mean is based on n = 3 replicates.

(34%), followed by loam (22%) and sandy loam (10%). Organic matter was highest in the sandy loam (1.4%) and lowest in sand (0.1%); amounts were 1.0% in the loam and 0.7% in the silty clay loam. Cation exchange capacity was highest in the silty clay loam (18.3%) and lowest in sand (1.4%).

Effect of Imidacloprid on Termite Feeding. The ANOVA results indicated that imidacloprid reduced termite feeding in sand (F = 799.3; df = 6, 14; P = 0.0001), sandy loam (F = 93.2; df = 6, 14; P = 0.0001), loam (F = 87.1; df = 6, 14; P = 0.0001), and silty clay loam (F = 25.1; df = 6, 14; P = 0.0001) (Table 2).

The effect of the chemical differed in the various soils even when the same concentration of Premise was used. In sand, feeding was reduced from 14.4 mg of filter paper in the control (untreated control) to ≤ 1.6 mg in the presence of ≥ 0.5 ppm Premise (Table 2). In contrast, the addition of 0.5 ppm Premise in sandy loam soils only reduced feeding from 17.4–14.0 mg and slightly increased feeding in loam soil (11.0–11.6 mg) and in silty clay loam soil (6.5–7.4 mg). At higher concentrations of Premise (≥5.0 ppm), reduced feeding was more evident. In sand, feeding was reduced from 0.9–0.0 mg, 3.1–1.4 mg in sandy loam, 1.7–0.0 mg in loam, and 0.9–1.0 mg in silty clay loam soils. There was no significant difference in consumption with Premise concentrations in sand ranging from 1 to 20 ppm and in the other soil types from 5 to 50 ppm (Table 2). At the labeled rate (50 ppm) of Premise 75 WP consumption was reduced 100, 100, 92, and 85% in sand, loam, sandy loam, and silty clay loam, respectively. Termite mortality 48-h after exposure to Premise 75 WP was negligible at all concentrations (also see 3-d mortality in Tables 3–6).

Effect of Imidacloprid on Termite Mortality. Mortality of termites differed when the same concentration of imidacloprid was added to the various soil types. At one-tenth the labeled rate of imidacloprid (5 ppm), percentage of mortality on day 21 in sand was 95.8% (Table 3), contrasted with 8.8% in sandy loam (Table 4), 5.6% in loam (Table 5), and 66.6% in silty clay loam (Table 6). Mortality comparable to that observed in sand required 50 ppm Premise in sandy loam (95.9%, Table 4) and 35–50 ppm in silty clay loam (96.4–93.3%, Table 6). The maximum concentration of Premise tested in loam soil resulted in only 81.6% mortality (Table 5). By day 21, there was no significant difference in termite mortality among the highest concentrations of Premise tested (25, 35, and 50 ppm) in sandy loam (Table 4) and loam soils (Table 5). A similar trend was noted in sand (Table 3) and silty clay loam soils (Table 6).

Effect of Treatment x Colony Interaction on Termite Mortality. The treatment x colony interaction was not significant in sand (F = 1.09; df = 5, 128; P = 0.3706) and loam (F = 0.46; df = 7, 173; P = 0.8635) but was significant in sandy loam (F = 2.36; df = 7, 173; P = 0.0253) and in silty clay loam (F = 3.32; df = 7, 173; P = 0.0024) (Table 7).

Further analysis of treatment and colony as a two-factor experiment in sandy loam showed that colony was a significant factor only at five and 50 ppm (Table 8). Thus, the variation of the data from sandy loam soil between colonies was not consistent over all concentrations. However, results from a similar analysis in silty clay loam indicated a significant difference between colonies at all concentrations (Table 9).

Discussion

Feeding inhibition and mortality experiments performed in this study demonstrated that imidacloprid

Table 3. Mortality of *R. flavipes* workers continuously exposed to Premise 75 WP-treated sand

Premise 75 WP concn, ppm	n	% mortality (mean ± SE) at day after exposure				
		3	7	10	14	21
0.0	24	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0d	0.0 ± 0.0d	0.0 ± 0.0e
0.1	24	0.0 ± 0.0c	1.0 ± 0.0bc	3.8 ± 0.0cd	5.1 ± 0.0cd	10.1 ± 0.0de
0.5	24	1.0 ± 0.0bc	11.3 ± 0.0ab	19.2 ± 1.0abc	25.2 ± 1.3c	38.4 ± 1.2cd
1.0	24	1.4 ± 0.0bc	7.8 ± 0.0abc	11.0 ± 0.0bcd	27.6 ± 1.1bc	51.9 ± 1.2bc
2.5	24	4.9 ± 0.0ab	20.5 ± 1.2a	38.2 ± 1.2ab	63.5 ± 1.4ab	84.5 ± 0.0ab
5	24	8.2 ± 0.0a	22.3 ± 1.0a	23.7 ± 1.0a	73.5 ± 1.1a	95.8 ± 0.0a
		F = 6.99	F = 5.80	F = 9.25	F = 17.05	F = 29.56
		df = 5, 129	df = 5, 129	df = 5, 129	df = 5, 129	df = 5, 129
		P = 0.0001	P = 0.0001	P = 0.0001	P = 0.0001	P = 0.0001

Means within each column followed by the same letter are not significantly different (Tukey HSD test [SAS Institute 1990]). Each mean is based on 600 termites (24 replicates × 25 termites per replicate).

Table 4. Mortality of *R. flavipes* workers continuously exposed to Premise 75 WP-treated sandy loam soil

Premise 75 WP concn, ppm	n	% mortality (mean ± SE) at day after exposure				
		3	7	10	14	21
0	24	0.0 ± 0.0ab	0.0 ± 0.0d	0.0 ± 0.0e	0.0 ± 0.0c	0.0 ± 0.0c
2.5	24	0.0 ± 0.0ab	0.0 ± 0.0d	0.0 ± 0.0e	0.0 ± 0.0c	1.4 ± 0.0c
5	24	0.0 ± 0.0ab	1.5 ± 0.0d	2.1 ± 0.0de	2.4 ± 1.0c	8.8 ± 0.0c
10	24	0.0 ± 0.0ab	4.7 ± 0.0cd	21.9 ± 1.6bcd	39.1 ± 1.9b	51.6 ± 1.7b
15	24	0.0 ± 0.0ab	5.6 ± 0.0bcd	12.7 ± 1.0cde	36.6 ± 1.5b	56.8 ± 1.8b
25	24	2.7 ± 0.0a	29.5 ± 0.0abc	42.9 ± 1.6abc	71.8 ± 1.4ab	92.6 ± 1.0a
35	24	1.5 ± 0.0ab	32.6 ± 0.0ab	58.3 ± 1.4ab	86.7 ± 1.1a	91.7 ± 1.0a
50	24	0.0 ± 0.0ab	38.6 ± 0.0a	63.3 ± 1.3a	92.7 ± 1.2a	95.9 ± 1.2a
		$F = 2.64$	$F = 9.64$	$F = 14.53$	$F = 30.89$	$F = 36.76$
		df = 7, 173	df = 7, 173	df = 7, 173	df = 7, 173	df = 7, 173
		$P = 0.0130$	$P = 0.0001$	$P = 0.0001$	$P = 0.0001$	$P = 0.0001$

Means within each column followed by the same letter are not significantly different (Tukey HSD test [SAS Institute 1990]). Each mean is based on 600 termites (24 replicates × 25 termites per replicate).

was active in a variety of soil types. Soil type influenced the bioavailability of imidacloprid; however, all the termiticide concentrations were at or below the manufacturers suggested final soil concentration (50 ppm).

Complete termite mortality in any of the soil types may not have occurred because the soil was oven-dried. This may have altered microbial populations and soil properties. In another study (Ramakrishnan et al. 1999), we demonstrated that imidacloprid enhanced the susceptibility of *R. flavipes* to a fungal entomopathogen, thus reducing the required chemical concentrations. In these experiments 100% mortality was achieved. Other studies have shown imidacloprid is an effective insecticide for a variety of insects (Elbert et al. 1990, 1991). However, these studies did not examine the effect of soil type on this chemical.

Differences in both feeding inhibition and mortality data in the various soils tested indicate there were significant interactions between the termiticide and the soils. Soil type was the primary variable in these experiments, therefore the difference probably resulted from soil properties that can alter the insecticide's bioavailability. Other studies have shown that organic carbon is the major soil property that can adsorb imidacloprid (Cox et al. 1998b). The organic matter content we recorded in this study for sand is 7–14 times lower than for the other soil types (Table 1). Hence, it is not unusual to find that soils with higher organic matter require 10–20 times more imidacloprid to achieve feeding inhibition comparable to sand (Table 2). These results are not unique, because other insecticides applied to moist soils show similar trends with increasing organic matter content (Harris 1966, 1972; Campbell et al. 1971).

The organic matter content in loam soil (1.0%) was lower than sandy loam (1.4%), but less mortality was observed in the former soil type (Tables 4 and 5). Imidacloprid could be influenced by other soil characteristics such as the biota and by structural components (e.g., silt and clay). Soil entomopathogens have been shown to act synergistically with imidacloprid on insects (Boucias et al. 1996; Quintela and McCoy 1997, 1998; Ramakrishnan et al. 1999). Clays also have been shown to contribute to the sorption of imidacloprid (Cox et al. 1998a, 1998b). Although the bioassay technique used in this study did not identify specific soil components affecting the performance of imidacloprid, our results suggest that chemical affinity of the active ingredient for portions of the soil matrix affected toxicant availability for biological interactions. This observation agrees with the work of Forschler and Townsend (1996) who conducted studies of several termiticides in different soil types. Smith and Rust (1993) also reported differences in toxicity and repel-

Table 5. Mortality of *R. flavipes* workers continuously exposed to Premise 75 WP-treated loam soil

Premise 75 WP concn, ppm	n	% mortality (mean ± SE) at day after exposure				
		3	7	10	14	21
0	24	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c
2.5	24	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0c	0.0 ± 0.0c	7.9 ± 0.0bc
5	24	0.0 ± 0.0b	1.6 ± 0.0ab	2.2 ± 0.0c	3.1 ± 0.0c	5.6 ± 1.2bc
10	24	0.0 ± 0.0b	2.0 ± 0.0ab	3.5 ± 0.0bc	8.7 ± 0.0bc	18.5 ± 0.0b
15	24	0.0 ± 0.0ab	4.9 ± 0.0ab	5.5 ± 0.0bc	9.3 ± 1.4bc	22.6 ± 1.0b
25	24	1.9 ± 0.0a	8.1 ± 0.0ab	18.1 ± 1.3ab	33.7 ± 1.4ab	70.0 ± 1.1a
35	24	1.3 ± 0.0a	17.2 ± 1.2a	24.9 ± 1.4a	42.2 ± 1.4a	77.2 ± 1.4a
50	24	1.3 ± 0.0a	7.8 ± 0.0ab	14.2 ± 0.0ab	26.2 ± 0.0ab	81.6 ± 1.0a
		$F = 8.25$	$F = 4.06$	$F = 6.20$	$F = 11.67$	$F = 22.94$
		df = 7, 173	df = 7, 173	df = 7, 173	df = 7, 173	df = 7, 173
		$P = 0.0001$	$P = 0.0004$	$P = 0.0001$	$P = 0.0001$	$P = 0.0001$

Means within each column followed by the same letter are not significantly different (Tukey HSD test [SAS Institute 1990]). Each mean is based on 600 termites (24 replicates × 25 termites per replicate).

Table 6. Mortality of *R. flavipes* workers continuously exposed to Premise 75 WP-treated silty clay loam soil

Premise 75 WP concn, ppm	*n*	% mortality (mean ± SE) at day after exposure				
		3	7	10	14	21
0	24	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0b	0.0 ± 0.0c	0.0 ± 0.0c
2.5	24	4.4 ± 0.0ab	16.3 ± 1.0b	26.8 ± 1.2a	36.2 ± 1.4b	42.0 ± 1.6c
5	24	3.2 ± 0.0bc	21.2 ± 1.2b	34.7 ± 1.4a	52.4 ± 1.7ab	66.6 ± 1.9bc
10	24	7.3 ± 0.0ab	28.4 ± 1.2ab	39.9 ± 1.4a	51.9 ± 1.4ab	69.4 ± 1.8bc
15	24	12.0 ± 0.0ab	36.1 ± 1.5ab	49.8 ± 1.4a	63.5 ± 1.6ab	75.5 ± 1.6abc
25	24	2.9 ± 0.0bc	19.6 ± 0.0ab	42.7 ± 1.3a	62.1 ± 1.8ab	76.2 ± 1.8abc
35	24	3.9 ± 0.0ab	21.9 ± 1.0ab	24.6 ± 1.0a	74.7 ± 1.1a	96.4 ± 0.0a
50	24	12.3 ± 0.0a	44.5 ± 1.3a	71.1 ± 1.4a	82.4 ± 1.3a	93.3 ± 0.0ab
		$F = 7.61$	$F = 7.67$	$F = 12.85$	$F = 16.70$	$F = 26.02$
		df = 7, 173	df = 7, 173	df = 7, 173	df = 7, 173	df = 7, 173
		$P = 0.0001$	$P = 0.0001$	$P = 0.0001$	$P = 0.0001$	$P = 0.0001$

Means within each column followed by the same letter are not significantly different (Tukey HSD test [SAS Institute 1990]). Each mean is based on 600 termites (24 replicates × 25 termites per replicate).

lency of termiticides tested in sand amended with cellulose or kaolin clay.

Termite source also should be considered when mortality experiments are conducted. Our data suggest that the termite colony combined with soil type can result in differences in mortality. Termite colony was a significant factor in the silty clay loam soil but not the other soils tested (Table 7). This may lead one to erroneously conclude that colony source effects mortality in only one soil type; however, there are confounding factors in the experimental design that may account for the differences observed. The confounding effects can be a combination of imidacloprid bioavailability (discussed above), colony growth status and colony-related susceptibility to imidacloprid. In our study, termites were collected and used for the experiments at different times of the year. Bioassays in silty clay loam were conducted in September, whereas the sand experiment was conducted in early July, the loam in August, and sandy loam in early October. Termite foraging is greatest when it is warm and is affected to a great deal by temperature and moisture (Haagsma and Rust 1995, Collins 1991). Bioassays in silty clay loam, conducted in early September, showed a difference in colony susceptibility at each level of imidacloprid tested (Table 9). Colony-dependent differences in xenobiotic detoxication capacity have been shown. The dark southern subterranean termite, *R. virginicus* (Banks), displayed a 2.7-fold difference in aldrin epoxidase activity among four colonies studied (Valles et al. 1998).

Data from constant exposure bioassays typically demonstrate trends in toxic effects of termiticides, but they do not measure termiticide performance in the field (Su et al. 1982, Forschler and Townsend 1996). Therefore, the data from our study cannot be directly extrapolated to the field situation. However, differences due to soil type were demonstrated. Further

Table 7. Factorial ANOVA of the effects of imidacloprid concentration and termite colony on the mortality of *R. flavipes* after a 21-d exposure to imidacloprid-treated soils

Soil type	Source	Sum of Squares	Mean Square	*F*	*P*
Sand[a]	Treatment (T)	94,001.8	18,800.3	29.56	0.0001
	Colony (C)	24.2	24.2	0.04	0.8456
	T × C	3,456.9	691.4	1.09	0.3706
	Trial	4,318.0	1,439.3	2.26	0.0842
	Error	82,034.9	635.9		
Sandy Loam[b]	Treatment (T)	166,892.3	23,841.8	36.76	0.0001
	Colony (C)	439.5	439.5	0.68	0.4115
	T × C	10,696.8	1,528.1	2.36	0.0253
	Trial	14,468.5	4,822.8	7.44	0.0001
	Error	112,193.6	648.5		
Loam[b]	Treatment (T)	100,727.1	14,389.6	22.94	0.0001
	Colony (C)	627.1	627.1	1.00	0.3188
	T × C	2,012.7	287.5	0.46	0.8635
	Trial	28,782.2	9,594.1	15.29	0.0001
	Error	108,534.2	627.4		
Silty Clay Loam[b]	Treatment (T)	102,938.8	14,705.5	26.02	0.0001
	Colony (C)	47,558.7	1,879.3	84.14	0.0001
	T × C	13,155.4	8,769.5	3.32	0.0024
	Trial	26,308.5	565.2	15.51	0.0001
	Error	97,785.7			

[a] df T = 6; C = 1; T × C = 5; trial = 3; error = 128.
[b] df T = 7; C = 1; T × C = 7; trial = 3; error = 173.

Table 8. Mortality of *R. flavipes* workers after 21 d of continuous exposure to sandy loam treated with Premise 75 WP

Premise 75 WP concn, ppm	% mortality (mean ± SE)		Critical statistics
	Colony A	Colony B	
0	0.0 ± 0.0e	0.0 ± 0.0d	
2.5	2.4 ± 0.0dex	0.0 ± 0.0dx	$F = 1.33$; df = 1, 19; $P = 0.2630$
5	25.6 ± 2.6cdex	0.0 ± 0.0dy	$F = 9.15$; df = 1, 19; $P = 0.0070$
10	37.1 ± 2.9bcdx	51.6 ± 3.8bcx	$F = 1.17$; df = 1, 19; $P = 0.2930$
15	61.9 ± 4.0abcx	66.3 ± 3.4cx	$F = 0.18$; df = 1, 19; $P = 0.6770$
25	90.1 ± 2.4ax	95.6 ± 1.3abx	$F = 0.28$; df = 1, 19; $P = 0.6026$
35	84.9 ± 1.9abx	96.5 ± 1.9abx	$F = 1.42$; df = 1, 19; $P = 0.2487$
50	81.6 ± 4.0abx	100.0 ± 0.0ay	$F = 7.91$; df = 1, 19; $P = 0.0111$
	$F = 14.98$	$F = 30.49$	
	df = 7, 85	df = 7, 85	
	$P = 0.0001$	$P = 0.0001$	

Means within columns (a–e) and rows (x and y) followed by the same letter are not significantly different (Tukey HSD test [SAS Institute 1990]). Each mean is based on 300 termites (12 replicates × 25 termites per replicate).

Table 9. Mortality of *R. flavipes* workers after 21 d of continuous exposure to silty clay loam treated with Premise 75 WP

Premise 75 WP concn, ppm	% mortality (mean ± SE)		Critical statistics
	Colony A	Colony B	
0	0.0 ± 0.0c	0.0 ± 0.0d	
2.5	73.0 ± 2.2bx	14.2 ± 2.7cdy	$F = 7.96$; df = 1, 19; $P = 0.0109$
5	91.7 ± 0.0ax	21.6 ± 2.9bcdy	$F = 21.91$; df = 1, 19; $P = 0.0002$
10	95.9 ± 1.4abx	31.9 ± 3.5abcdy	$F = 14.69$; df = 1, 19; $P = 0.0011$
15	89.6 ± 2.1abx	57.5 ± 3.8abcx	$F = 4.31$; df = 1, 19; $P = 0.0516$
25	99.1 ± 0.0ax	36.1 ± 3.9abcy	$F = 24.51$; df = 1, 19; $P = 0.0001$
35	99.9 ± 0.0ax	87.7 ± 2.0ay	$F = 5.47$; df = 1, 19; $P = 0.0304$
50	100.0 ± 0.0ax	77.2 ± 1.9aby	$F = 19.27$; df = 1, 19; $P = 0.0003$
	$F = 36.85$ df = 7, 85 $P = 0.0001$	$F = 7.82$ df = 7, 85 $P = 0.0001$	

Means within columns (a–e) and rows (x and y) followed by the same letter are not significantly different (Tukey HSD test [SAS Institute 1990]). Each mean is based on 300 termites (12 replicates × 25 termites per replicate).

laboratory studies are required in fresh soils to indicate precisely which components of the soil matrix affect imidacloprid. Laboratory bioassays of termiticides offer valuable insight into understanding the activity of a chemical and in increasing the efficiency of applied termiticides.

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