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Effects of non-repellent termiticides on the tunneling and walking ability of the eastern subterranean termite (Isoptera: Rhinotermitidae)

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Abstract

BACKGROUND: Non-repellent insecticides, including fipronil and indoxacarb, are becoming increasingly important for soil treatments to manage the eastern subterranean termite, *Reticulitermes flavipes* (Kollar). The effects of these insecticides on termite walking and tunneling behavior could significantly reduce their efficacy against colonies.

RESULTS: Groups of *R. flavipes* were exposed to several concentrations of commercial formulations of fipronil and indoxacarb, and the ability of treated termites to tunnel in soil and walk was assessed. Increasing insecticide concentration resulted in a reduction in the ability of *R. flavipes* to walk, tunnel and form tunnel branches; the importance of these effects on the use of non-repellent insecticides is discussed.

CONCLUSION: Exposure of *R. flavipes* to 1, 10 or 50 mg L^{-1} of fipronil or 50, 100 or 200 mg L^{-1} of indoxacarb significantly reduced termite walking and tunneling and the number of tunnel branches. Distance walked (ca 73 mm) by untreated control termites did not change over time for at least 16 h after treatment; control termites formed ca 150 cm of tunnels with ca 40 branches.

Keywords: fipronil; indoxacarb; eastern subterranean termite; *Reticulitermes flavipes*

1 INTRODUCTION

Subterranean termites (Rhinotermitidae) forage for cellulosic food and frequently attack structural wood. In the United States, the eastern subterranean termite, *Reticulitermes flavipes* (Kollar), is undoubtedly the single most economically important termite species, partly because of its wide geographical distribution relative to other species.[1]

Wooden structures were typically protected with repellent compounds until the relatively recent development of non-repellent termiticides, which are becoming increasingly more popular. Effective use of repellent compounds requires relatively more rigorous procedures to minimize the occurrence of untreated gaps in the chemical barrier under and around structures.[2] Uniform application is imperative with repellent compounds, but much less critical with non-repellent ones. Environmental concerns associated with many of the conventional repellent chemicals have also led to the use of baits and non-repellent liquid alternatives.[3] Desirable qualities of slow-acting, non-repellent compounds include their permissive nature, which allows termites to continue tunneling in treated soils for considerable periods before they become intoxicated and immobilized and die.[4,5] These compounds allow treated termites ample time and opportunity to interact with and thereby contaminate unexposed nestmates in locations that may be far away from the treated area.

Fipronil and indoxacarb are two non-repellent insecticides that have generated considerable interest for termite management. Fipronil is currently one of the most commonly used non-repellent compounds in the termite control industry.[6] It is a phenylpyrazole that functions by blocking the γ-aminobutyric-acid-gated chloride channel of the insect nervous system.[7] Indoxacarb is classified as a compound with reduced toxicity,[8] and successful field trials against termites have drawn attention to this oxadiazine; its mode of action is as a voltage-dependent sodium channel blocker.[3] The efficacy and non-repellence of these two compounds,[4,9] the horizontal transfer of fipronil[5,6,10–12] and transfer of indoxacarb[4] have all been demonstrated in laboratory studies with termites.[4,5,13,14] Effects of non-repellent compounds on the foraging range and activity of subterranean termites were studied under both laboratory and field conditions.[15,16] The influence of soil compaction,[17] moisture gradients[18] and the presence of sound or decayed wood on tunneling behavior and

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ability of termites has also been examined.[19] There is still little information, however, on the effects of fipronil and indoxacarb on the tunneling behavior and walking ability of termites. These behaviors likely affect the area protected by these compounds.

The purpose of this study was to describe the tunneling behavior and walking ability of eastern subterranean termites treated with fipronil and indoxacarb. A second objective was to determine the effects of insecticide concentration and the post-exposure period on termite mobility. It is hypothesized that tunneling behavior and walking ability will be negatively correlated with insecticide concentration.

2 EXPERIMENTAL METHODS

2.1 Termites

Groups of eastern subterranean termite workers were collected in Auburn-Opelika (Lee County, AL, 32° 36′ 00.34″ N, 85° 28′ 57.48″ W) in March and April 2008 using underground traps described by Hu and Appel.[20] Traps consisted of covered open-bottom plastic buckets (18 cm in height, 13 cm in diameter) provisioned with corrugated cardboard rolls (15 cm in height, 11 cm in diameter). Termite collections were brought to the laboratory, and termites were extracted by gently tapping the cardboard rolls to allow termites to drop onto a moist paper towel in a tray. Freshly collected fifth- to seventh- instar termites were used for studies.

2.2 Chemicals

Formulated fipronil [Termidor SC (9.1% AI); BASF Corp., Research Triangle Park, NC] and indoxacarb [Indoxacarb 150 SC (15% AI); E.I. Dupont, de Nemours and Company, Wilmington, DE] were used in this study. Formulated materials were used because formulations are applied to control termites, not just the active ingredients. Plastic petri dishes (1.5 cm in height, 5.2 cm in diameter) provisioned with Whatman No. 1 filter paper (Whatman International Ltd, Maidstone, UK) of the same diameter served as treatment chambers. Calculated amounts of fipronil and indoxacarb were diluted in water and used to prepare various concentrations of insecticide-treated filter paper. Filter paper treated with 1, 10 and 50 mg L^{-1} of fipronil and 50, 100 and 200 mg L^{-1} of indoxacarb was used in the treatments. Concentrations of fipronil and indoxacarb were selected on the basis of previous studies by the authors,[21,22] and to reflect the relative toxicities of the two compounds. The major objective of this study was to determine the concentration effects of each insecticide on tunneling and walking. Preliminary studies of these insecticides on filter paper resulted in the choice of the concentrations. For indoxacarb, 45, 90 and 180 g L^{-1} represents half, full and double label rates of the insecticide respectively; 50, 100 and 200 ppm rates of indoxacarb were used instead to compensate for the fact that termites tunneling in soil come into contact with treated soil on all sides (i.e. dorsal, ventral and lateral), whereas the filter paper provided direct contact with the termite body parts in contact with it. The properties of these insecticides on filter paper may not exactly match those in soil medium. Use of filter paper helped to ensure a more uniform exposure of termites and also reduced injury to termites during transfer from the treatment chamber to the tunneling/walking arena. Preliminary studies indicated that no activity would be recorded when termites were exposed to fipronil concentrations of >50 mg L^{-1}. Filter paper treated with distilled water was the control treatment. Treated filter papers were air dried in a hood for 24 h and later moistened with 0.4 mL of distilled water prior to the test.

2.3 Test for tunneling of workers

Soil was obtained from the immediate surroundings of a field termite colony in Auburn (Lee County, AL). The soil was sieved using a 25 mesh (0.71 mm) screen to remove debris and sterilized in an autoclave (Fisher Scientific®) at 80 °C for 24 h, 2 days prior to the experiment. Distilled water was added to the soil to achieve 10% moisture (w/w). Moist soil weighing 1.075 g was used to fill a cylindrical plastic container (9 cm in height, 25 cm in diameter) to a height of 2 cm, resulting in a bulk density of approximately 1.09 g cm^{-3}.

In a preliminary test, 100 workers of at least fifth larval instar were exposed to filter paper treated with 200 mg L^{-1} of indoxacarb (0.4 mL of insecticide solution per filter paper) for 10, 20, 40 and 60 min. Termites exposed to filter paper treated with distilled water served as a control, and all treatments were replicated 3 times. Treated termites were transferred into transparent plastic cups (∼30 mL; Fill-rite Corp., Newark, NJ); these cups were inverted to deposit termites on soil in the middle of cylindrical transparent plastic arenas (9 cm in height, 25 cm in diameter), each of which constituted an experimental unit. Cups were pressed gently into the soil to drive the rim to a depth of ≈1 cm. The entire arena was then covered with a lid and sealed with cellophane to prevent loss of moisture. Experimental units were placed on the transparent glass sheet of an observation platform. The observation platform consisted of a rectangular wooden framework with a transparent glass top and mirror inclined at an appropriate angle beneath the glass top to enable direct observation of activities at the base of each experimental unit. Tunneling activity and behavior were recorded at hourly intervals for the first 16 h, after which observations were taken at 6 h intervals until complete cessation of tunneling. Progress in tunnel construction was tracked by tracing the path of the tunnels on the bottom of the transparent arena using colored markers. The labeling system used by Hedlund and Henderson,[23] and later by Pitts-Singer and Forschler,[24] was used to define the elaborate network of tunnels. Tunnels were defined on the basis of their origin with respect to the site of introduction of termites. Primary tunnels originate from the introduction site and terminate at the opposite edge of the arena; secondary tunnels branch off primary tunnels, and tertiary tunnels branch off secondary tunnels.

Based on preliminary results, 10 min of exposure to insecticide deposits of the concentrations described above was selected to test effects of fipronil and indoxacarb on the tunneling ability of eastern subterranean termites. Observation and marking of tunnels were conducted at the same time intervals as those described in the preliminary test. The test was concluded when tunneling in all treatments had ceased for 12 h. Termites in the arenas that were alive but immobilized were placed on a sheet of Whatman No. 1 filter paper (55 mm in diameter; Whatman International Ltd, Maidstone, UK) to determine whether they were still capable of walking.

2.4 Test of walking ability of insecticide-intoxicated subterranean termites

The test arena consisted of a petri dish (13.6 cm in diameter) lined with Whatman No. 1 filter paper (12.5 cm in diameter; Whatman international Ltd, Maidstone, UK). An 8.8 cm diameter circle was drawn on the filter paper with the aid of a compass and a black ink ballpoint pen (Paper Mate Write Bros. Med. Pt., Sanford, Oak Brook, IL). Paper Mate ballpoint pen ink contains 2-phenoxyethanol, a compound that elicits termite trail-following.[25]

Groups of ten termites were exposed to various concentrations of insecticide-treated filter paper for 10 min before their individual walking ability was measured in the test arena. Termites were allowed ca 15 s to find the ink trail. A marker was used to record the start and end points of termite movement on the ink circle for 60 s. A thread was placed along the path covered by the termites, and the distance walked was determined by measuring the thread with a ruler. Tests were repeated at 2 h intervals until termites became immobilized. A separate test arena was used for each treatment (i.e. concentration and exposure time combination).

2.5 Statistical analysis

Tunneling data were $\log_{10}$ transformed to stabilize the variance, but the untransformed data are reported (mean $\pm$ SE). Regression analysis was used to determine the effects of insecticide concentration on the tunneling ability of termites post-treatment. The cumulative number of branches was estimated by non-linear regression (SigmaPlot, v.11),[26] using the rectangular hyperbolic model cumulative number of branches

$$\mathrm{CNB} = \frac{\mathrm{CNB}_{max} \cdot \mathrm{h}}{t_{\mathrm{CNB}/2} + \mathrm{h}}$$

where CNB_{max} is the horizontal asymptote (maximum CNB), $t_{\mathrm{CNB}/2}$ is the hour at which 50% of CNB_{max} is reached and h denotes hour. Estimates and standard errors of CNB_{max} and $t_{\mathrm{CNB}/2}$ were generated and compared by overlap of the estimates ± 2 SE. Linear regression was used to describe the change in CNB over time if the rectangular hyperbolic model was not appropriate.

Walking distance data were analyzed separately for each insecticide using a randomized split-plot design;[27] concentration was the main plot, and post-exposure time was the subplot. Means were separated using Tukey's honestly significant difference test;[27] a significance level of $P = 0.05$ was used.

3 RESULTS

3.1 Tunneling behaviors

Irrespective of the concentration or type of insecticide used, termites tunneled to the base of the arena and then constructed tunnels that radiated out from that central introduction site and towards the periphery of the arena. Termites at the tunnel head picked up soil particles reversed or turned around to deposit soil particles on the tunnel wall away from the tunnel head. Other termites gained access to the tunnel head, either by squeezing past termites that were reversing or by walking back in a forward direction after turning around in enlarged portions of the tunnel. Reversing termites eventually turned around at enlarged areas of the tunnel. To create enlarged areas, tunneling was performed at the tunnel head by two or three termites at the same time, or tunneling was done at different parts of the tunnel head by different termites. Transport of soil particles from the tunnel head in enlarged portions was achieved by a simple turn-around and subsequent deposition of soil particles at earlier portions of the tunnel. Sometimes termites lingered at the tunnel head, causing termites immediately behind them to hit their posterior. This action usually elicited reversing or a turn-around and exiting of the tunnel head by the lead termite. Termites behind the lead termite(s) apparently were cued by reversing or returning termites and reversed until the lead termite(s) arrived at enlarged areas where the next termite could squeeze past it to the tunnel head.

In the untreated control treatment, tunneling declined after a number of tunnels reached the periphery of the test arena; this occurred in spite of the presence of tunnel-free areas. Subsequent movement of termites consisted mainly of walking inside the constructed tunnels. One cause of branching (in the insecticide treatments) was the occlusion of tunnel heads by moribund termites, thereby preventing more active termites in older portions of the tunnel from reaching the tunnel head. The tandem posture of termites during excavation necessitated the withdrawal of the lead termite(s) and replacement by termites next in line. Failure to leave tunnel heads necessitated the branching of tunnels by relatively active workers located behind moribund individuals. The majority of moribund termites were found at tunnel heads at the end of the study. Insecticide-induced reduction in physical ability may be partly responsible for branching of tunnels as an adaptation to minimize the expenditure of energy in tunneling.

An interesting observation among the indoxacarb-treated termites was the 'lone ranger' phenomenon, in which tunneling was carried out at one or two tunnel heads by a single termite long after other termites had stopped tunneling owing to ataxia or moribundity. This kind of tunneling typically involved removal of soil particles from the tunnel head, walking in reverse, deposition of soil particles in older portions of the tunnel and walking forward to pick up another particle from the tunnel head. These 'lone rangers' very seldom turned around to walk forward when they transported soil particles away from the tunnel head. Another interesting observation was that, irrespective of treatment (i.e. control or insecticide treated), once termites made contact with the bottom of the arena, the majority of them tunneled from an upside-down position, with their dorsum in contact with the bottom of the arena. Termites only walked upright inside the tunnels when they were not engaged in gallery extension or when tunneling had ceased.

3.2 Quantitative indices of tunneling capacity

In the preliminary study, the total length of tunnels constructed by termites treated with 200 mg L^{-1} of indoxacarb was significantly less than that in the controls ($P < 0.05$); tunnel lengths decreased by 54.17 ± 22.41, 55.86 ± 22.01, 70.98 ± 14.01 and 89.21 ± 5.26% in termites exposed to indoxacarb for 10, 20, 40 and 60 min respectively. Time to cessation of tunneling and the total number of branches were unaffected by duration of exposure to indoxacarb (Table 1). The total length of tunnels constructed remained similar for exposure durations of 10–40 min but declined when termites were treated with indoxacarb for 60 min. Based on the results of the preliminary test (Table 1), a 10 min exposure was selected to test the effects of insecticide concentration on tunneling; this enabled a conservative estimate of the duration of treatment required to incapacitate termites.

Untreated termites constructed more and longer tunnels ($P < 0.05$) compared with those in the fipronil (Fig. 1) and indoxacarb (Fig. 2) treatments. The concentration of indoxacarb did not affect the total number of branches constructed. The reduction in total tunnel length from 45.60 ± 3.35 cm in the 1 mg L^{-1} fipronil treatment to 1.68 ± 1.13 cm in the 50 mg L^{-1} fipronil treatment and the reduction from 55.05 ± 4.87 cm in the 50 mg L^{-1} indoxacarb treatment to 22.60 ± 8.16 cm in the 200 mg L^{-1} indoxacarb treatment were both statistically insignificant ($P > 0.05$). The 15.25 ± 1.44 tunnels constructed in the 1 mg L^{-1} fipronil treatment were statistically similar to the

Table 1. Tunneling ability of eastern subterranean termite workers exposed to filter paper treated with 200 mg L-1 of indoxacarb (mean ± SE) (total number of workers = 100; number of replicates = 10)[a]

Exposure time (min)	Total length of tunnels (cm)	Time to cessation of tunneling (*h*)	Number of branches
0	31.67 ± 2.17 a	∞	7.33 ± 0.46 a
10	14.33 ± 6.51 ab	8.70 ± 1.39 a	5.33 ± 0.46 a
20	13.53 ± 6.27 ab	9.03 ± 1.18 a	7.00 ± 0.48 a
40	9.73 ± 5.21 ab	8.73 ± 1.21 a	8.00 ± 1.02 a
60	3.63 ± 1.99 b	7.13 ± 1.85 a	3.33 ± 0.21 a
F	4.64	0.36	1.02
P	0.0223	0.7869	0.4429

[a] Means followed by different letters within the same column differ ($P < 0.05$).

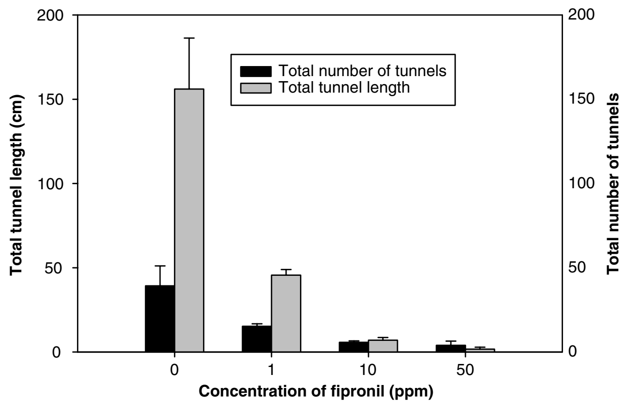

Figure 1. Effect of fipronil concentration on the total length of tunnels and number of branches constructed by eastern subterranean termite workers.

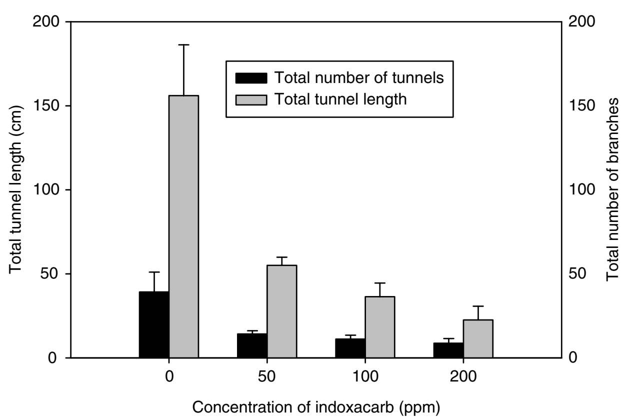

Figure 2. Effect of indoxacarb concentration on the total length of tunnels and number of branches constructed by eastern subterranean termite workers.

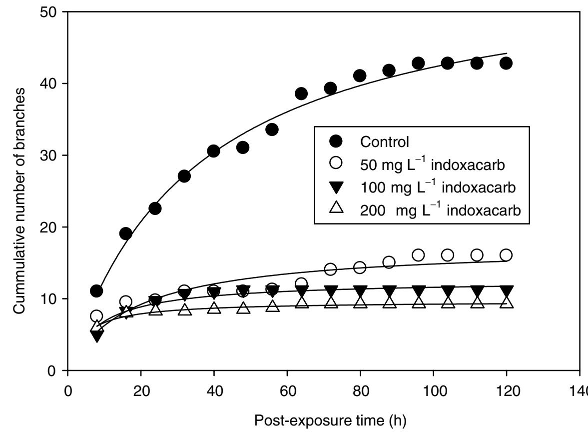

Figure 3. Effect of indoxacarb concentration on the total number of branches constructed by eastern subterranean termite workers at various times after exposure.

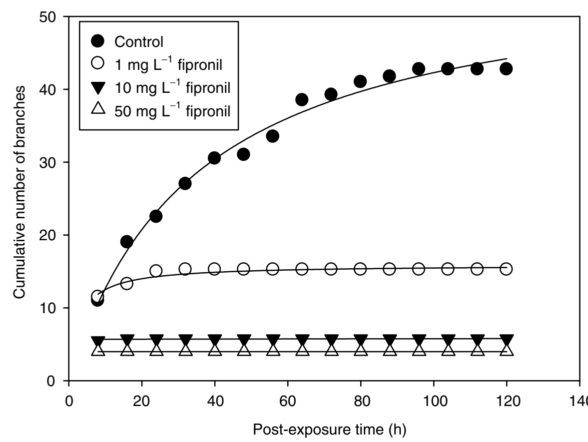

Figure 4. Effect of fipronil concentration on the total number of branches constructed by eastern subterranean termite workers at various times after exposure.

4.00±2.42 tunnels constructed in the 50 mg L^{-1} fipronil treatment ($P > 0.05$).

Increase in the number of branches was achieved either by branching of existing tunnels or by construction of new primary tunnels. Time trends show that the number of branches constructed by untreated termites increased as a rectangular hyperbolic function until ≈96 h after introduction into the test arena (Table 3, Figs 3 and 4).

The maximum number of cumulative branches was 57.12±1.57, and half of that maximum occurred at 35.08 ± 2.75 h. There were significant ($P > 0.05$) concentration effects in CNB_{max} and $t_{CNB/2}$ for both the indoxacarb (Fig. 3) and fipronil (Fig. 4) treatments. The CNB_{max} for indoxacarb ranged from 17.51 ± 1.06 at 50 mg L^{-1} to 9.65 ± 0.10 at 200 mg L^{-1}. Similarly, the $t_{CNB/2}$ declined with increasing concentration from 18.04 ± 4.42 h to 4.45 ± 0.41 h for 50 and 200 mg L^{-1} respectively (Table 2).

The number of new branches constructed in the 50 mg L^{-1} indoxacarb treatment was more than the number of new branches constructed in the 100 and 200 mg L^{-1} treatments (Fig. 3). For fipronil, the CNB_{max} and $t_{CNB/2}$ also declined with increasing concentration (Fig. 4). The CNB_{max} for fipronil ranged from 15.89 ± 0.15 at 1 mg L^{-1} to 4.00 ± 0.00 at 50 mg L^{-1}. The $t_{CNB/2}$ also declined with increasing concentration from 2.70 ± 0.32 h to 0 h for 1 and 50 mg L^{-1} respectively (Table 2). The number of branches leveled off as early as the first 2 h in the 10 and 50 mg L^{-1} fipronil treatments, whereas a barely noticeable number of branches were still added until 25–30 h after exposure to 1 mg L^{-1} of fipronil (Fig. 4). The number of

Table 2. Non-linear regression coefficients relating the cumulative number of branches (CNB) for fipronil- and indoxacarb-treated termites to time after exposure (ten replicates per treatment)

Treatment	Concentration (mg L^{-1})	CNB_{max}	$t_{CNB/2}$	*F*	*P*	r^2
Control	0	57.12 ± 1.57	35.08 ± 2.75	1020.21	<0.0001	0.986
Fipronil	1	15.89 ± 0.15	2.70 ± 0.32	113.36	<0.0001	0.889
	10	5.78 ± 0.01	0.33 ± 0.05	40.89	<0.0001	0.740
	50	4.00 ± 0.00	0[a]	–	–	–
Indoxacarb	50	17.51 ± 1.06	18.04 ± 4.42	53.68	<0.0001	0.790
	100	12.55 ± 0.30	8.32 ± 1.22	135.83	<0.0001	0.906
	200	9.65 ± 0.10	4.45 ± 0.41	225.75	<0.0001	0.941

[a] Not significant ($P > 0.05$).

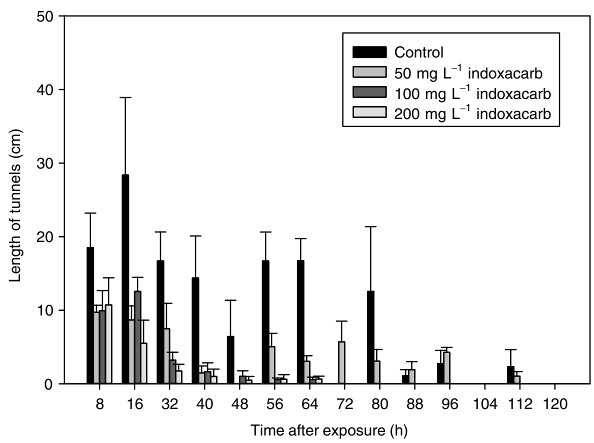

Figure 5. Effect of indoxacarb concentration on the length of tunnels constructed by eastern subterranean termite workers at various times after exposure.

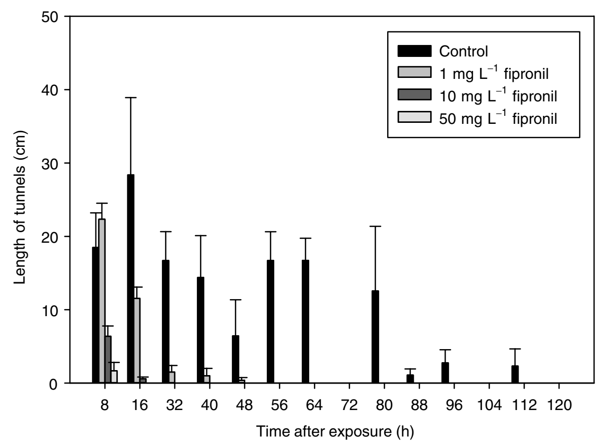

Figure 6. Effect of fipronil concentration on the length of tunnels constructed by eastern subterranean termite workers at various times after exposure.

branches constructed in the 1 mg L^{-1} fipronil treatments was more than the number of branches constructed in the 10 and 50 mg L^{-1} fipronil treatments (Fig. 4). Interestingly, the CNB_{max} of the 1 mg L^{-1} fipronil and the 50 mg L^{-1} indoxacarb treatments were equivalent (≈15.3); however, half of that maximum was reached earlier for the 1 mg L^{-1} fipronil treatment (2.7 h) than for the 50 mg L^{-1} indoxacarb treatment (18.04 h).

Time trends in tunnel construction (Fig. 5) show that untreated termites extended their tunnels more than the indoxacarb-treated termites at each time interval until ≈88 h when tunneling ceased or almost ceased because ≈40% of branches had reached the edge of the arena. Tunneling ceased with 100 and 200 mg L^{-1} of indoxacarb 64–72 h after treatment, whereas termites in the 50 mg L^{-1} indoxacarb treatment ceased tunneling 112–120 h after treatment. Distance tunneled by termites declined with time after exposure to indoxacarb ($F = 6.16$, df = 7, $P < 0.0001$); however, concentration was not significant ($P > 0.05$), even though reduction in tunneling distance at higher concentrations was a consistent trend. Tunneling was faster for untreated termites ($F = 99.99$, df = 1, $P < 0.0001$). Untreated termites constructed a greater number of branches compared with indoxacarb-treated individuals ($F = 80.92$, df = 1, $P < 0.0001$), but concentration and time after treatment did not have significant effects ($P > 0.05$). Untreated termites extended their galleries by a significantly greater distance ($F = 158.42$, df = 1, $P < 0.0001$) and number of branches ($F = 102.41$, df = 1, $P < 0.0001$) and at a faster pace ($F = 158.68$, df = 1, $P < 0.0001$) compared with fipronil-treated termites (Fig. 6). Distance tunneled declined with time after exposure ($F = 6.04$, df = 7, $P < 0.001$). Termites in the 10 and 50 mg L^{-1} fipronil treatments tunneled similar distances ($P > 0.05$), but those in the 1 mg L^{-1} fipronil treatments tunneled significantly greater distances ($F = 8.54$, df = 2, $P = 0.0004$). The number of branches constructed was similar at various times post-exposure ($P > 0.05$) for the 10 and 50 mg L^{-1} treatment, but the 1 mg L^{-1} fipronil treatments had more tunnels ($F = 13.96$, df = 2, $P < 0.0001$). Termites treated with various concentrations of fipronil tunneled at significantly different rates ($F = 8.55$, df = 2, $P = 0.0004$). Among fipronil treatments, tunneling was fastest in termites exposed to paper treated with 1 mg L^{-1} of fipronil. Tunneling in the 10 and 50 mg L^{-1} fipronil treatments ceased by 32 and 16 h after treatment respectively, whereas tunneling in the 1 mg L^{-1} treatments continued until 56 h after treatment. Untreated controls tunneled until 112 h, when ≈30% of tunnels radiating from the centre of the arena had reached the edge.

Approximately 65% of insecticide-treated termites that appeared to be immobilized at the end of the tunneling test were capable of walking ≈20 cm min^{-1}.

3.3 Walking ability

Distance walked by untreated termites remained fairly constant ($P > 0.05$) throughout the study, but it declined 8 h after the treatment of termites with 100 mg L^{-1} of indoxacarb and 1 mg L^{-1}

Table 3. Walking distance per min (cm) of indoxacarb-treated termites (mean ± SE) (ten replicates per treatment)[a]

	Control	Indoxacarb		
Time after exposure (*h*)	0 mg L^{-1}	50 mg L^{-1}	100 mg L^{-1}	200 mg L^{-1}
0	78.68 ± 3.42 a	88.41 ± 3.54 a	77.95 ± 3.81 a	73.7 ± 3.19 a
2	73.87 ± 2.22 a	87.6 ± 2.37 a	78.17 ± 5.66 a	58.43 ± 3.80 b
4	74.78 ± 2.32 a	77.94 ± 1.54 b	72.14 ± 6.74 a	31.75 ± 3.26 c
6	73.83 ± 1.67 a	69.13 ± 2.91 b	61.22 ± 9.30 ab	12.43 ± 3.06 d
8	71.90 ± 3.15 a	59.33 ± 3.78 c	41.92 ± 7.40 bc	0.00 ± 0.00 e
10	73.02 ± 2.71 a	50.13 ± 3.52 d	21.35 ± 6.62 cd	0.00 ± 0.00 e
12	69.95 ± 2.49 a	31.27 ± 5.91 e	15.40 ± 5.56 cd	0.00 ± 0.00 e
14	71.34 ± 2.81 a	10.3 ± 3.17 f	0.00 ± 0.00 d	0.00 ± 0.00 e
16	72.34 ± 2.49 a	0.5 ± 0.50f	0.00 ± 0.00 d	0.00 ± 0.00 e
F	0.99	138.82	24.66	165.29
P	0.44	<0.0001	<0.0001	<0.0001

[a] Means followed by different letters within the same column differ ($P < 0.05$).

Table 4. Walking distance per min (cm) of fipronil-treated termites (mean ± SE) (ten replicates per treatment)

	Control	Fipronil		
Time after Exposure (*h*)	0 mg L^{-1}	1 mg L^{-1}	10 mg L^{-1}	50 mg L^{-1}
0	78.68 ± 3.42 a	77.26 ± 3.60 a	79.08 ± 2.82 a	73.93 ± 4.05 a
2	73.87 ± 2.22 a	69.85 ± 3.37 a	40.7 ± 4.03 b	29.14 ± 1.74 b
4	74.78 ± 2.32 a	65.36 ± 5.56 a	15.4 ± 4.64 c	7.2 ± 2.72 c
6	73.83 ± 1.67 a	62.27 ± 5.41 a	0.00 ± 0.00 d	0.00 ± 0.00 d
8	71.90 ± 3.15 a	29.61 ± 10.27 b	0.00 ± 0.00 d	0.00 ± 0.00 d
10	73.02 ± 2.71 a	16.64 ± 9.08 bc	0.00 ± 0.00 d	0.00 ± 0.00 d
12	69.95 ± 2.49 a	7.40 ± 3.82 bc	0.00 ± 0.00 d	0.00 ± 0.00 d
14	71.34 ± 2.81 a	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 d
F	0.99	26.80	150.58	210.20
P	0.44	<0.0001	<0.0001	<0.0001

[a] Means followed by different letters within the same column differ ($P < 0.05$).

of fipronil (Tables 3 and 4). Untreated termites never ceased walking during the entire 120 h study, but complete cessation of walking was observed 14 h after treatment in both insecticide treatments.

4 DISCUSSION

The experimental design did not allow an unfettered view of the specific role of the various mouthparts in the excavation process, but the activities observed generally matched previous descriptions.[17,28–32] Termites excavated soil particles instead of squeezing between pores. Positive geotaxis may explain the tendency to tunnel from an inverted position. This orientation places the dorsum against a more solid surface than the ventral surface. It is also possible that this orientation was induced by the nature of the arena; the hard plastic base of the arena differs from conditions in the field. On the other hand, this orientation might reveal a tendency of termites to place their dorsum on a relatively harder surface than their ventral side. This behavior may have major implications for the orientation of tunnels and requires further study.

Tunneling behavior and the reduced capacity of intoxicated termites to walk reflected their decline in health; as reported by Strack and Myles,[33] intoxication effects were clearly observable without having physically to interfere with the test arena. Mobility of termites is an important determinant of the amount of horizontal transfer of toxicants, which in turn affects the rate and magnitude of termite colony mortality. When tunneling capacity is compromised, as was the case among insecticide-treated termites in the present study, mobility of termites is restricted to walking in tunnels constructed prior to intoxication. It is well established that horizontal transfer of toxicants increases with increasing population density and contact frequency between individual termites.[34] Probability of contact between treated and untreated termites declines with declining LT_{90} value of an insecticide. Lower LT_{90} values associated with higher insecticide concentrations cause termites to become immobilized and lose their abilities to tunnel and interact with nestmates earlier. Tunnel construction involves the expenditure of energy, which weakens the termites and accelerates the onset of moribundity, particularly for insecticides that interfere with energy production; this may explain why most of the moribund termites were found at the tunnel heads. Hu[35] observed that insecticide-exposed foraging termites usually managed to walk back to the colony central nest before losing equilibrium; this behavior resulted in positioning of intoxicated foragers where they could receive care from their nestmates. The different observations of the present study could be due to the relatively small groups of workers used rather than an entire colony. According to Shelton and Appel,[36] single untreated eastern subterranean termites move at a constant, relatively steady pace, with very short pauses from time to time; this is in stark contrast to the behavior under intoxication observed in the present study.

Branching of termite tunnels may occur as a means of increasing the area or volume of excavation to maximize food-searching activity. Branching may also reflect an innate tendency of foraging termites to achieve a certain amount of coverage (i.e. tunnel density) of their surroundings. The importance of the latter factor was demonstrated by the ≈40% decline in tunneling among untreated controls after a number of tunnels reached the wall of the arena. This density, which might be a function of both termite population size and foraging area, seems to reduce the tendency of foragers to continue tunneling. Foraging termites in field situations are, however, not restricted by arena walls and may not exhibit this behavior but may reduce their tunneling activities in response to other cues or events such as the discovery of a rich source of food or a large rock or another solid impenetrable object such as concrete or cement blocks. Eastern subterranean termite workers have, however, been reported to continue foraging without altering their speed significantly after discovering a food source.[37] These factors agree with the suggestion of Pitts-Singer and Forschler[24] that termites have an innate tendency to optimize the construction of tunnels to access new sources of food. The authors defined an optimal pattern as one that minimized search overlap but maximized the area searched using the number of tunnel branches constructed. Tunnels constructed by eastern subterranean termites in this study were long and narrow; an adaption that may increase the probability of finding other sources of food underground.[37]

The significant reduction in cumulative tunnel length when termites were exposed to 10 and 50 mg L^{-1} of fipronil, compared with those treated with 1 mg L^{-1} of fipronil, confirms the findings of Yeoh and Lee,[38] who conducted a similar experiment using continuous exposure to treated soil rather than limited exposure to filter paper. The number of tunnel branches stabilized as early as the first 2 h in the 10 and 50 mg L^{-1} fipronil treatments, whereas very few branches were added until 25–30 h after exposure to 1 mg L^{-1} of fipronil. Termites that had ceased tunneling and walking in the arena were able to move considerable distances when removed and placed on filter paper. This is possibly because walking demands less energy than tunneling, and individual termites behave differently to those in groups in soil.

There have been a number of suggestions on how to improve the performance of contact insecticides in soil. These include alteration of the residual and amount of active contact; active contact is defined as a method by which insects contact insecticides by tunneling through a band of insecticide-treated soil.[39] The author suggested the incorporation of chemicals that elicit or enhance digging into formulations of contact insecticides, resulting in greater contact with insecticides and reductions in the amount of insecticides necessary to achieve the same degree of control as conventional insecticide formulations. Termites in the present study acquired toxicants through treated filter paper instead of moving through treated soil, but termites exhibited the expected trends in tunneling nonetheless.

The arena used in the present study is less restrictive compared with the testing tubes described by Remmen and Su.[13] Artificial tubes are desirable if the objective is to measure unidirectional tunneling. While branching of tunnels and other tunneling behaviors are restricted in testing tubes, the reduction in tunneling in the untreated controls when tunnels reach the walls of the area in the present study also constitutes environmental inhibition.

Tunnel density (length and number of branches per unit area) seems to have an influence on the tendency of foragers to continue tunneling; higher tunnel densities seem to cause a decline in tunneling activity. The effect of olfactory gradients and stigmergic cues as determinants of tunnel orientation were not examined in this study, so their significance cannot be ruled out.[40–42]

Tunneling and walking ability of intoxicated termites are important factors that affect their potential to transfer toxicants to naive nestmates. Based on the results presented here and in other studies, increased insecticide concentrations and exposure times reduced tunneling and walking and would therefore also likely reduce horizontal transfer to other colony members. These results imply that greater exposure to insecticides by more colony members should occur if lower concentrations and reduced exposure times can be used. As precise amounts of liquid insecticides can be applied by injection, it might be possible to spot-treat multiple locations, resulting in a pattern of treated and untreated areas. Tunneling termites would encounter treated soil, from which they would obtain some insecticide, but then be able to leave the treated area and tunnel further without greater exposure (dose or time). In this way, termites might be able to forage further and enhance horizontal transfer while still ultimately obtaining a lethal dose. It is very important to conduct further studies to test the interaction of termites treated with indoxacarb and fipronil and naive termites in actual soil treatments to determine effects on tunneling, walking distance and ultimately colony management.

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