Catnip Essential Oil as a Barrier to Subterranean Termites (Isoptera: Rhinotermitidae) in the Laboratory

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ABSTRACT The essential oil of catnip, *Nepeta cataria* (Lamiacae) was evaluated for behavioral effects on two populations of subterranean termite, *Reticulitermes flavipes* (Kollar) and *R. virginicus* (Banks) (Isoptera: Rhinotermitidae). The catnip essential oil contained ≈36: 64 *E,Z*-nepetalactone and *Z,E*-nepetalactone, respectively. The time to 50% dissipation (DT_{50}) of the isomers in sand was dependent on dose, and ranged from 5.7 to 12.6 d for the *E,Z*-isomer and 7.7–18.6 d for the *Z,E*-isomer. For *R. flavipes*, the 24-h topical LD_{50} value was ≈8200 μg/g termite. The 24-h fumigation LC_{50} value for *R. flavipes* was between 36 and 73 μg/ml air, and the 7-d fumigation LC_{50} value was between 14 and 36 μg/ml air. Exposure of *R. virginicus* to treated sand resulted in a 24-h LC_{50} value (95% F.L.) of 84 (67.6, 112) μg/cm^2 and a 7-d LC_{50} value of 21.1 (16.4, 26.8) μg/cm^2; for *R. flavipes* these values were 63.2 (53.7, 73.9) and 44.4 (34.6, 58.1) μg/cm^2, respectively. Vertical tunneling through treated sand was eliminated at 500 ppm for *R. virginicus* and at 250 ppm for *R. flavipes*. Horizontal tunneling was stopped at 250 ppm for *R. virginicus* and reduced at doses above 250 ppm for *R. flavipes*. Although tunneling ceased in these tests, mortality was not high, indicating that the termites avoided the treated sand. Efficacy of catnip oil was equivalent to other monoterpenoids reported in the literature.

KEY WORDS nepetalactone, catnip, termite, soil barrier, *Reticulitermes*

WOOD CONTINUES TO BE a popular and economical raw material for the building of structures, desirable because of its availability, ease of working, and esthetic appeal. Wood and other wood products are subject to attack by many species of subterranean termites; in the United States, mostly *Reticulitermes* spp. and *Coptotermes* spp. (Isoptera: Rhinotermitidae). Proper protection of wood products from termites will lessen the demand for new wood materials. This will increase the sustainability of forested lands, benefiting the environment by reducing human manipulation of forest ecosystems and benefiting the economy by keeping wood and wood products inexpensive. Chemical treatment is the most common method of protection, either through the use of wood preservatives or treatment of the soil adjacent to wood structures. Some soil termiticide active ingredients, such as chlordane and chlorpyrifos, have lost their registrations in the United States. New compounds are being investigated to fill the void left by discontinued products, and registered active ingredients include imidacloprid, fipronil, chlorfenapyr, and several pyrethroids. The Forest Service, United States Department of Agriculture, conducts efficacy testing for companies pursuing termiticide registration in the U.S. About three new termiticidal formulations are tested per year, with a new active ingredient being tested about once in every 2 yrs. Natural compounds from plants, bacteria and fungi may provide valuable new leads for the development of new commercial products. Certain natural products are exempt from United States Environmental Protection Agency registration because of reduced risk to humans, pets, and the environment.

Catnip, *Nepeta cataria* L. (Lamiaceae), is a perennial herb known for its intoxicating effect on cats. The essential oil of catnip is composed almost entirely of two isomers (the *Z,E*- and *E,Z*-forms) of the monoterpenoid nepetalactone [5,6,7,7a-tetrahydro-4,7-dimethylcyclopenta[c]pyran-1-(4aH)-one] and is repellent to insects such as the German cockroach (Peterson et al. 2002), the house fly (C.J.P., unpublished data) and the yellowfever mosquito (Peterson 2001). Recent work has shown that catnip essential oil is repellent to the Eastern subterranean termite, *Reticulitermes flavipes* (Kollar), when applied to filter paper (Haenke et al. 2002). Similar natural monoterpenoid compounds, such as citral, citronellal, eugenol, geraniol, and nerol are toxic and repellent to termites (Cornelius et al. 1997) and nootkatone, a sesquiterpenoid, displayed toxic and repellent activity as well (Zhu et al. 2001a, b). Bläske and Hertel (2001) found

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that several botanical insecticides were effective as chemical barriers to termites. Activation of octopaminergic receptors is a possible mode of action for monoterpenoids (Kostyukovsky et al. 2002). The current study examines the effect of catnip essential oil-treated sand on termite mortality and distribution within laboratory apparatuses. The potential of using this oil as a termite barrier was evaluated by measuring termite tunneling through treated sand. As well, we examined the longevity of nepetalactone in treated sand.

Materials and Methods

Termites. Two subterranean termite species were collected from the Choctaw Wildlife Management Area of the Tombigbee National Forest near Ackerman, MS, 10 d before the tests commenced. The populations, separated by ≈3 km, were collected from infested pine logs. Sections of the logs, containing termites, were placed in metal garbage cans according to species and taken to the laboratory. The cans were covered with metal lids and stored at ambient conditions in the laboratory and termites were removed as needed. Termites were identified to species by examination of soldiers as *R. virginicus* (Banks) and *R. flavipes* (Kollar) (Gleason and Koehler 1980, Scheffrahn and Su 1994). The average weight of an individual worker termite of *R. virginicus* ($n = 15$) was 1.60 ± 0.01 mg and *R. flavipes* ($n = 15$) was 3.30 ± 0.07 mg. Although many prealates were observed, alates were not available from these populations for identification. The USDA Forest Service, Wood Products Insect Research Unit, Starkville, MS, has retained voucher specimens of both species used in this study.

Essential Oil. The essential oil of catnip (neat) was purchased from Kong Pet Products, Golden, CO. Analysis by high performance liquid chromatography, described below, determined that the oil was 36: 64 *E,Z*- to *Z,E*-nepetalactone.

High Performance Liquid Chromatography (HPLC). All chromatographic analyses were conducted on a Waters 2695 liquid chromatography system, using a Waters Symmetry C_{18} column (4.6 × 75 mm, 3.5 μm particle size) (Waters Corporation, Milford, MA). Injection volume was 10 μl, and the mobile phase was isocratic at 60: 40 methanol: water at a flow rate of 0.5 ml/min. Detection was accomplished with a Waters 996 photodiode array detector, scanning from 210 to 260 nm, with quantitation of the isomers based on peak area at 225 nm. An external standard of nepetalactone was used to construct the standard curve of each isomer.

Topical Toxicity. This assay was conducted following the method of Valles and Woodson (2002a). Filter paper circles (cut with a paper punch) were placed in individual wells of a 96-well microplate. Five microliters of distilled water was placed in each well. Each of 12 termite workers were treated on the abdomen with 0.5 μl of the appropriate acetone dilution of catnip essential oil by using a Hamilton PB-600 microapplicator (Hamilton Co., Reno, NV), and each termite was placed singly into individual wells of the microplate. Each plate was wrapped with a moist paper towel and placed in a plastic box with a lid. The boxes were placed in an unlit incubator at 25 ± 1°C at ≈70% RH. Mortality was recorded at 24 h and at 7 d. Three replicates of 12 termites were conducted and the test was run twice. Five doses resulting in >0 but <100% mortality were used to calculate LD_{50} values by using probit analysis on SAS (SAS Institute 1996), after correction for control mortality by Abbott's equation (Abbott 1925).

Fumigation Assay. A 150-ml glass jar with a screw cap was used as a fumigation chamber. A 4.25-cm diameter piece of filter paper was placed in the bottom of the jar and 150 μl of distilled water was applied to the filter paper. Twenty termite workers were added to the jar and a second piece of 4.25-cm filter paper was treated with 200 μl of the appropriate acetone dilution of catnip oil. The acetone was allowed to dry for 5 min in a fume hood, and then was placed over the mouth of the jar, and then the jar was tightly capped. The jars were placed in an unlit incubator at 25 ± 1°C at ≈70% RH. Three replicates of 20 termites were conducted. The 24 h and 7 d treatments were set up separately.

Contact Toxicity. This method is based on that reported by Kard et al. (1989). Sand was dried overnight at 120°C to remove water, and then was treated by applying 20 ml of the appropriate acetone dilution of catnip essential oil to 100 g of sand. After application, the sand was mixed on a jar roller for 5 min, the sand was removed from the jar and placed on a 25-cm petri dish for 1 h in a fume hood to remove the acetone. Agar was poured into 60 × 15 mm petri dishes to a depth of 2 to 3 mm and allowed to cool. Treated sand (1 g) was evenly poured on top of the agar. Doses of catnip oil were: 5, 10, 25, 50, 100, 250, 500, 750, and 1000 ppm (mass/mass) in the sand. Ten worker termites were introduced into each petri dish and each dish was covered. The termites were observed at 15-min intervals for 6 h, then hourly for two additional hours, then again at 24 h and 7 d. Blank and solvent controls were used, and the test had three replicates. LC_{50} values were calculated by probit analysis on SAS (SAS Institute 1996) after correction for control mortality by Abbott's equation (Abbott 1925).

Longevity. Excess sand from the solvent, 10, 100, 500, 750, and 1000 ppm doses of the petri dish toxicity tests was subjected to HPLC analysis. Twenty grams of sand were extracted with 20 ml methanol, filtered and injected into the HPLC for quantitation (as described above). This served as a check of nominal dose. A standard curve was constructed at identical conditions and the standard curve was used to determine the concentration of each isomer in the sand. Jars of sand were capped and sealed with Parafilm (American National Can Company, Chicago, IL) and stored in an unlit incubator at 25 ± 1°C at ≈70% RH. The test was replicated three times. At 1-wk intervals, 20 g portions of sand were removed from the jars and extracted with 20 ml methanol, followed by analysis on HPLC. Sampling continued until the treated sand was depleted. The data were used to determine the rate of

disappearance of the individual nepetalactone isomers. The Proc Mixed function for repeated measures on SAS was used to determine significant effects (SAS Institute 1996).

Vertical Barrier Assay. Three doses (100, 250 and 500 ppm) were selected for use in this test. Each test apparatus consisted of a 2.5-cm diameter by 25-cm long glass test tube. A 2 × 1 × 1 cm block of southern yellow pine sapwood was placed at the bottom of the test tube. The test tube was filled with a 50: 50 (vol:vol) mixture of sand and vermiculite substrate to a depth of 6 cm, and 5.5 ml of distilled water was added. One hundred grams of sand was treated as described above in the contact toxicity assay. Treated sand was added to a depth of 6 cm (≈35.5 g sand) on top of the sand-vermiculite mixture and 5.5 ml distilled water was added (≈15% moisture by weight). A top layer of sand and vermiculite substrate was added to the test tube to a depth of 6 cm and a 1.5-cm wooden cube was pressed 0.5 cm into the substrate. The top substrate was moistened with 5.5 ml of distilled water. Eighty worker termites plus one soldier were placed on top of the upper 6 cm substrate. Each tube was covered with a piece of Parafilm, and the tubes were labeled with the appropriate test information. The test was replicated five times for each concentration (including acetone control) and termite species. The tubes were placed in an unlit incubator at 25 ± 1°C at ≈70% RH. After 1 wk, the depth of visible tunneling in the treated sand was measured, the tubes were emptied and the number of termites recovered from each tube was recorded. The distance tunneled, and the number of termites recovered, were subjected to analysis of variance (ANOVA) (SAS Institute 1996).

Horizontal Barrier Assay. Three doses (100, 250, and 500 ppm) were selected for use in this test. Modifications of the methods of Forschler (1994) and Bläske and Hertel (2001) were used to determine the effect of catnip treated sand on horizontal tunneling of termites. This method used a zone of untreated sand, the "Introduction" zone, a "Barrier" zone of treated sand, and another untreated, or "Protected" zone, on the other side of the Introduction zone in a transparent 13.5 × 12.75 cm box (Fig. 1A). Sand (100 g) was treated as described in the petri dish toxicity assay. Paper cards were placed in the box to divide it into equal thirds. Once the solvent had evaporated, 100 g of the treated sand, 100 g untreated sand for the Introduction zone, and 100 g untreated sand for the Protected zone, were added to the boxes in a way to provide a barrier of treated sand through the middle of the box. The depth of the sand was ≈1 cm. Each zone was moistened with 20 ml of distilled water (20% moisture by weight) and the paper cards were removed. One 2 × 1 × 1 cm block of southern yellow pine sapwood was placed in the sand in both the Introduction and Protected zones ≈1.5 cm from the edge, and a small amount of sand was excavated around the blocks so that the termites had access to the bottom of the box. Termites (200 workers plus two soldiers) were placed in the Introduction zone on the wood block. The boxes were covered with lids, sealed with Parafilm, and then placed in an unlit incubator at 25 ± 1°C at ≈70% RH. A random number table was used to randomize the position of the Introduction zone, to the right or to the left. Five replicates were conducted for each concentration and for the acetone controls for both species. After seven days, the termite tunnels were examined by viewing the underside of the boxes to determine if the termites had penetrated the treated sand barrier. The undersides of the boxes were photocopied to document the visible tunnels on the bottom of the box. Visible tunneling may not reflect total tunneling, because the termites may construct tunnels not visible from below. Surviving termites were excavated and counted from each of the three zones in each box (Introduction zone, Barrier zone, and Protected zone). The photocopied tunnels and galleries were traced onto transparency film, photographed with a digital camera, converted to grayscale, and analyzed by ImagePro (version 3.2, Media Cybernetics, Silver Spring, MD) for total visible area excavated by the termites. Percentage survival (total for the entire box) and percentage of area excavated were transformed by the arcsine of the percentage and analyzed by ANOVA ($\alpha = 0.05$). Percentage recovered and percentage area excavated from each zone were arcsine transformed and analyzed individually by ANOVA (SAS Institute 1996).

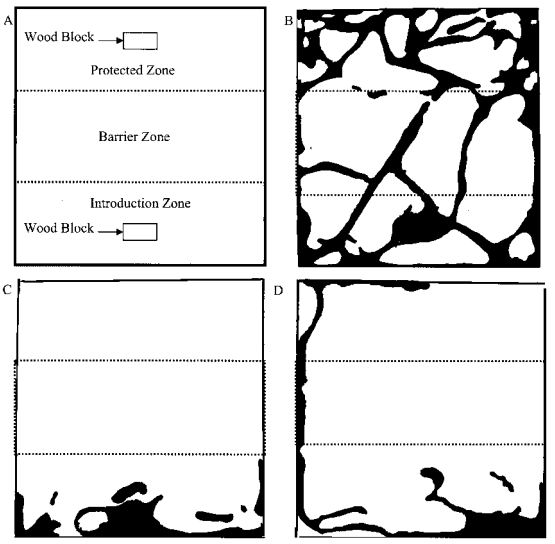

Fig. 1. Transparency tracings of visible termite tunnels in the horizontal tunneling assay. Dotted boxes approximate the location of the treated barrier. (A) Schematic of box layout, (B) Control treatment for *R. flavipes*, (C) prevention of *R. flavipes* tunneling through 250 ppm barrier, (D) breach of 250 ppm barrier along side of box by *R. flavipes*.

Results and Discussion

HPLC. The oil was found by HPLC to consist of a >98% mixture of the nepetalactone isomers, with only minor peaks observed for other (unidentified) components. Base-line separation of the isomers was ac-

complished. The ratio of isomers was 36: 64 *E,Z- : Z,E*-nepetalactone. *E,Z*-Nepetalactone eluted at 13.88 min (standard deviation = 0.246 min) and Z,E-nepetalactone eluted at 16.54 min (standard deviation = 0.30 min). The λ_{max} values for the *E,Z*- and the *Z,E*-isomer were 227.9 nm and 220.8 nm, respectively. Absorbance at 225 nm was used to quantify all peaks in the longevity analysis.

Topical Toxicity. *R. virginicus* was depleted by the behavioral assays, so LD_{50} determination was not possible. For *R. flavipes*, the LD_{50} value (95% Fiducial Limits) was 8200 (7600, 8900) μg oil/g termite. One-week LD_{50} values were not determined because of high control mortality, because of either desiccation or fungal growth. This microwell assay is new, and to our knowledge has only appeared twice in the literature (Valles and Woodson 2002a, b). The advantage of such an assay is that it separates the termites from one another, and group grooming behavior, which can exaggerate toxicity, is eliminated (Valles and Woodson 2002b). This assay has not previously been used for a natural product, so it is not possible to compare our results with those of previous studies. Valles and Woodson (2002a) report LD_{50} values for *Coptotermes formosanus* Shiraki of ≈37–78 μg/g termite for chlordane, ≈4–6 μg/g termite for chlorpyrifos, and ≈1–2 μg/g termite for cypermethrin in this assay. In other topical assays, none of which used a natural product, a topical LD_{50} value of 68.61 μg/g to *R. flavipes* was determined for GX071 (sulfluramid) (Su and Scheffrahn 1988), and 1.78 μg/g for mirex (Su and Scheffrahn 1991). For *R. flavipes*, LD_{50} values may be as low as 0.01 μg/g (deltamethrin) (Su and Scheffrahn 1990). The high LD_{50} value for the catnip oil may reflect the volatility of the compound. All monoterpenoids are volatile to some degree, especially at such low amounts, and they likely evaporate off the surface of the insect before a sufficient amount is absorbed. Also, monoterpenoids may have lower intrinsic activity as acute topical toxicants as compared with other compounds.

Fumigation Assay. As with the microwell assay, *R. virginicus* was exhausted by the behavioral assays. Data sufficient for the calculation of an LC_{50} value for *R. flavipes* were not obtained in the fumigation test. A 24-h LC_{50} value can be expected to lie between 36 and 73 μg oil/ml air. All insects were dead within 24 h at 146 μg oil/ml air, 85% were dead at 109 μg oil/ml air, 90% at 73 μg oil/ml air, 13% at 36 μg oil/ml air and 0% at 14 μg oil/ml air (control mortality was 2%). The 7-d data jumped from 0% mortality at 14 μg oil/ml air to 100% mortality at 36 μg oil/ml air (control mortality was 2%). Reliable LC_{50} calculations are not possible with such a range in mortality. A preliminary fumigation test on a different *R. flavipes* population (data not shown) jumped from 5% mortality in 24 h at 1.5 μg oil/ml air to 85% mortality at 7.3 μg oil/ml air (control mortality was 2%).

Many natural products, including monoterpenoids, have been used in various fumigation assays with termites. Bläske and Hertel (2001) found that a dose of 276 μg/ml air of isoborneol and cedarwood oil caused 100% mortality of *R. santonensis* within 48 h. Higher activity was seen for *C. formosanus* with citral, geraniol and eugenol, with 2.2, 2.1 and 0.27 μg/ml air, respectively, resulting in 100% mortality in 48 h (Cornelius et al. 1997). Catnip oil toxicity is within the range of these monoterpenoids and essential oils.

Contact Toxicity. Data collected from both populations were corrected for control mortality (from 0 to 7%) by using Abbott's equation (Abbott 1925) and LC_{50} values (95% Fiducial Limits) were calculated. *R. virginicus* had a 24-h LC_{50} value of 84 (67.6, 112) μg/cm^2, and a 7-d value of 21.1 (16.4, 26.8) μg/cm^2. *R. flavipes* was roughly as susceptible as *R. virginicus* in 24 h and had an LC_{50} value of 63.2 (53.7, 73.9) μg/cm^2 but was more tolerant at 7 d, with an LC_{50} value of 44.4 (34.6, 58.1) μg/cm^2. At 83 μg/cm^2, >24 h was required to kill one-half of the *R. virginicus* specimens, while for *R. flavipes* one-half of the insects were dead in 5 h at this dose. Fumigation as well as contact both contributed to the observed mortality in this test.

A test of catnip oil on filter paper with *R. flavipes* was conducted by Haenke et al. (2002), and 7570 μg/cm^2 catnip oil caused 93% mortality in 30 min, 757 μg/cm^2 caused 68% mortality in 1 h, and 75.7 μg/cm^2 caused 29% mortality in 4 h. For some monoterpenoids, 24-h LD_{50} values for *Odontotermes brunneus* ranged from 16 to 37 μg/cm^2, but other monoterpenoids were not active up to 350 μg/cm^2 (Sharma and Raina 1998). Cedarwood oil and isoborneol caused 100% and 53% mortality, respectively, in 48 h of *R. santonensis* at 119 μg/cm^2 (Bläske and Hertel 2001) and 100% mortality in 48 h was observed at ≈60 and 106 ppm in sand for eugenol and geraniol, respectively (Cornelius et al. 1997). The data observed in this test for catnip oil are consistent with those observed for other monoterpenoids. Kard et al. (1989) found that soil treated with pyrethroid insecticides at 11.1 μg/cm^2 and aged for 1.5–2 yr caused 100% moribundity in *R. virginicus* in <1 h.

Longevity. HPLC analysis of the sand extract at time = 0 d determined the applied doses of each isomer. No nepetalactone was recovered in the controls, and the extracts of the 10 ppm sand treatment were below the level of quantitation. In no case was 100% of the nominal dose recovered, which is expected for a volatile compound such as nepetalactone. Individual isomers were recovered at about the same percentage recovery at each dose. Recovered dose increased with increasing nominal dose. At 100 ppm, 31.1 and 38.0% of the nominal doses were recovered for *E,Z*- and *Z,E*-nepetalactone, respectively, this increased to 67.7 and 71.9% for the 500 ppm dose, 79.5 and 85% for the 750 ppm dose, and 85.8 and 87.5% at the 1000 ppm dose. Table 1 reports the ppm extracted from the sand at $t = 0$ and three additional time points over three replications.

Proc Mixed on SAS for repeated measures of the HPLC data for dissipation of *Z,E*-nepetalactone over time found significance because of dose ($F = 74.29$; $df = 3$; $P < 0.0001$), time ($F = 25.94$; $df = 3$; $P < 0.0001$) and the dose × time interaction ($F = 3.01$; $df = 9$; $P = 0.0179$). For *E,Z*-nepetalactone, significance was seen

Table 1. Concentration (ppm ± SEM) of nepetalactone isomers remaining in treated sand initially and at weekly intervals (averages of three replications)

Time (d)	0	10	100	500	750	1000
			E,Z-Nepetalactone; Nominal Dose of Oil[a]			
0	ND	ND	11.2 ± 4.8	122 ± 20.6	215 ± 15.8	309 ± 39.3
7	ND	ND	1.6 ± 1.6	70.7 ± 19.9	134 ± 23.4	218 ± 24.9
14	ND	ND	ND	35.4 ± 14.4	103 ± 11.8	128 ± 14.3
21	ND	ND	ND	23.5 ± 4.5	39.8 ± 14.5	61.9 ± 13.9
			Z,E-Nepetalactone; Nominal Dose of Oil[a]			
0	ND	ND	24.3 ± 7.6	230 ± 32	408 ± 28.6	560 ± 64.5
7	ND	ND	9.9 ± 8.0	154 ± 35.2	286 ± 34.5	430 ± 40.0
14	ND	ND	1.6 ± 1.6	103 ± 32.9	258 ± 14.4	332 ± 20.5
21	ND	ND	ND	98.3 ± 1.0	160 ± 33.3	260 ± 28.6

ND, below level of detection/quantitation.
[a] ≈36: 64 of the nominal dose is *E,Z*-:*Z,E*-nepetalactone, respectively.

for dose ($F = 53.35$; df = 3; $P < 0.0001$), time ($F = 38.48$; df = 3; $P < 0.0001$), and the dose × time interaction ($F = 5.58$; df = 9; $P = 0.0006$). The dissipation of each isomer best fit the linear model at doses of 500 ppm and higher ($r^2 > 0.9327$ and 0.8876 for *E,Z*- and *Z,E*-nepetalactone, respectively, with higher r^2 values at the higher doses).

The time to 50% dissipation (DT_{50}) of each isomer was calculated from the linear equations for the dissipation of each isomer, at one-half the recovered dose at time = 0. The DT_{50} for each compound was dose–dependent (Fig. 2A). Dose dependence of DT_{50} values was seen for chlorpyrifos (Racke et al. 1994), and this may be because of an antimicrobial effect at high doses (Tu 1995). *E,Z*-Nepetalactone had a DT_{50} of 5.7 d at 100 ppm, 10.9 d at 500 ppm, 12.5 d at 750 ppm, and 12.6 d at 1000 ppm. *Z,E*-Nepetalactone DT_{50} values were 7.7 d at 100 ppm, 15.4 d at 500 ppm, 17.2 d at 750 ppm, and 18.6 d at 1000 ppm. This increase in DT_{50} because of dose fit a logarithmic model with r^2 values of 0.9885 and 0.9998 for *E,Z*- and *Z,E*- nepetalactone, respectively (Fig. 2A). The DT_{50} values of *E,Z*-nepetalactone were ≈68–74% that of *Z,E*-nepetalactone. The rate of dissipation (slope of the linear curve) was higher (more negative) for the *Z,E*-isomer (Fig. 2B). This isomer dissipated faster (in terms of ppm/d) but required a longer time to reach one-half of its initial recovered dose. As well, the rate of dissipation was dependent on initial dose.

Nepetalactone dissipation characteristics are consistent with those observed for monoterpenoids by other researchers. Zhu et al. (2001b) found that monoterpenoids lost their effectiveness as repellents after ≈6 d, but the sesquiterpenoid nootkatone was effective for >24 d. Based on microbial CO_2 emission, Vokou and Margaris (1988) estimated that volatile plant oils and monoterpenoids persist in the soil for >15 d. Other natural products, such as azadirachtin A,

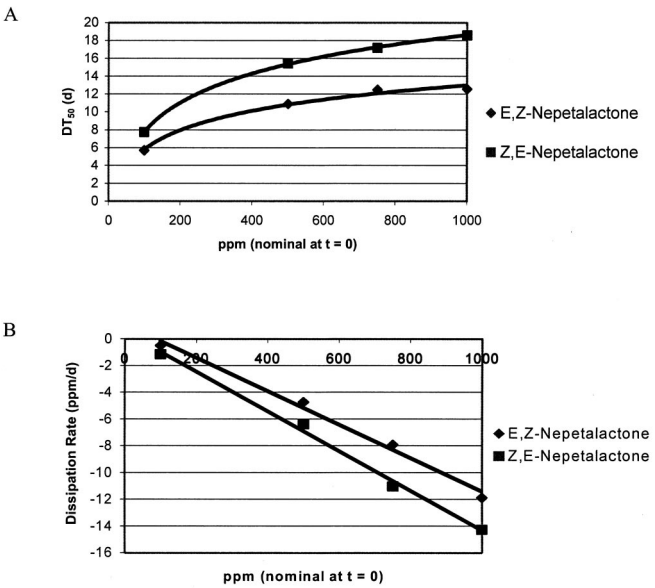

Fig. 2. (A) DT_{50} values of nepetalactone increasing as a function of initial dose, while (B) dissipation rate also increases with initial dose.

Table 2. Distance tunneled and percentage of termites recovered (mean ± SEM) in the vertical barrier assay (averages of five replications)

	R. virginicus		*R. flavipes*	
	Distance tunneled	% Recovered	Distance tunneled	% Recovered
Control	6 ± 0	58 ± 10	6 ± 0	73 ± 18
100 ppm	4.3 ± 0.7	83 ± 3	4.2 ± 1.2	55 ± 22
250 ppm	0.2 ± 0.2	56 ± 17	0 ± 0	96 ± 2
500 ppm	0 ± 0	92 ± 2	0 ± 0	72 ± 18

a compound from the neem tree, had DT_{50} values in water of 25–29 d, and, similar to our study, the DT_{50} values were dose dependent. Formulated products dissipated significantly more slowly than technical grade azadirachtin A (Thompson et al. 2002b). A fungal toxin, the methyl ester of fusaric acid, had a half-life from 6.2 to 47 d, depending on temperature, soil moisture and soil type (Vischetti and Esposito 1999). Spinosad, a microbial natural product, had a soil half-life in the absence of light of 9–17 d (Thompson et al. 2000) and a half-life in leaf litter from 2 to 12.4 d (Thompson et al. 2002a).

Of compounds used in termite control, chlorpyrifos had a soil half-life from ≈30 to >720 d (Racke et al. 1994) or 315–462 d (Baskaran et al. 1999), depending upon dose, soil type and temperature. Imidacloprid had a half-life of 990-1230 d at rates recommended for termite control (Baskaran et al. 1999). Fipronil, when incorporated into the soil, had a half-life of 90–220 d (Rhône-Poulenc, 1996).

Vertical Barrier Assay. The overall model for depth of tunneling related to dose and species was significant ($F = 31.22$; df = 7, 32; $P < 0.0001$). The depth of tunneling into the treated sand was significantly affected by catnip oil dose ($F = 72.80$; df = 3; $P < 0.0001$), but not by species ($F = 0.06$; df = 1; $P = 0.8119$) and the dose × species interaction was not significant ($F = 0.02$; df = 3; $P = 0.9963$). No significance was detected for the number of termites recovered from the tubes ($F = 1.36$; df = 7, 32; $P = 0.2569$). Means (±SEM) for depth and percentage of insects recovered are reported in Table 2. In the control groups, the sand was completely penetrated (tunneling measured 6 cm) by the termites in all units for both species. The average depth of visible tunneling decreased with an increase in dose. No tunneling was observed at 500 ppm for *R. virginicus*, or at 250 or 500 ppm for *R. flavipes*. The percentage of termites recovered from the tubes was not dose dependent. This indicates that the decrease in tunneling was not a result of mortality.

Horizontal Barrier Assay. The overall model for percentage of visible area excavated from the entire tray (all three zones together) was significant ($F = 27.56$; df = 7,32; $P < 0.0001$), and significance was seen for dose ($F = 49.01$; df = 3; $P < 0.0001$) and species ($F = 39.75$; df = 1; $P < 0.0001$) but the dose × species interaction was not significant ($F = 2.04$; df = 3; $P = 0.1282$). For *R. virginicus*, the average visible area excavated for the five replications decreased from 16.4 (±1.4)% in the control to 5.4 (±0.5)% at 250 and 500 ppm (Table 3). There were no observed penetrations of the treated sand at doses of 250 and 500 ppm, and, at these doses, the entire excavation was limited to the Introduction zone. In one case, however, termites were recovered from a zone in which no visible ex-

Table 3. Percentage of areas excavated and the number of termites recovered (± SEM) from each of the three zones and the whole box in the horizontal barrier assay (averages of five replications)

ppm	Introduction	Barrier	Protected	Total
R. virginicus		Percentage of area excavated		
0	26.0 ± 0.4	7.0 ± 1.8	16.2 ± 3.0	16.4 ± 1.4
100	24.7 ± 1.2	4.9 ± 1.1	10.8 ± 1.4	13.5 ± 0.7
250	15.4 ± 0.9	0 ± 0	0 ± 0	5.4 ± 0.4
500	15.6 ± 1.6	0 ± 0	0 ± 0	5.4 ± 0.5
		Number of termites recovered (of 200)		
0	59.4 ± 19.8	10.0 ± 4.1	101.6 ± 22.5	171 ± 6.2
100	119 ± 9.9	12.2 ± 3.9	29.8 ± 11.0	161 ± 3.0
250	132 ± 16.3	0 ± 0	0 ± 0	132 ± 16.3
500	80.6 ± 27.5	2.4 ± 2.4	0 ± 0	83 ± 27.8
R. flavipes		Percentage of area excavated		
0	33.0 ± 2.1	12.1 ± 1.6	32.3 ± 5.1	25.7 ± 2.5
100	37.9 ± 2.1	10.4 ± 1.6	12.4 ± 1.3	20.7 ± 0.9
250	26.1 ± 2.9	2.3 ± 1.0	3.4 ± 1.8	11.0 ± 1.7
500	20.8 ± 2.9	0.9 ± 0.6	2.5 ± 1.6	8.1 ± 1.5
		Number of termites recovered (of 200)		
0	78.8 ± 11.0	24.0 ± 5.5	68.2 ± 18.1	171 ± 4.3
100	133 ± 3.3	34.6 ± 9.6	11.6 ± 4.5	179 ± 3.9
250	153 ± 9.3	8.8 ± 2.7	0.6 ± 0.6	162 ± 7.4
500	129 ± 8.1	5.8 ± 2.8	2.8 ± 2.1	137 ± 5.0

cavation was measured (the Barrier zone of the 500 ppm dose). For *R. flavipes*, the total visible area excavated from the boxes declined from 25.7 (±2.5)% in the control to 8.1 (±1.5)% in the 500 ppm dose, and on average no zone for any dose was free of excavation. In two individual boxes at 250 ppm and three at 500 ppm, however, no excavations from the Barrier or Protected zones were observed, and in those boxes where excavations did occur, the termites had tunneled along the side of the box through the treated barrier (Fig. 1). Bläske and Hertel (2001) observed tunneling along the sides also. Edge effects are known to play a part in termite distribution, and termites make use of fixed objects in the soil as guides (Pitts-Singer and Forschler 2000).

As dose increased, the average area excavated from each zone decreased for both species. The amount excavated from the Introduction zone decreased from 26 (±0.4) to 15.6 (±1.6)% for *R. virginicus* and from 33 (±2.1) to 20.8 (±2.9)% for *R. flavipes*, although there was a slight increase at 100 ppm. Excavation from the Barrier zone decreased from 7.0 (±1.8) to 0% and from 12.1 (±0.6) to 0.9 (±0.6)% for *R. virginicus* and *R. flavipes*, respectively, and excavation from the Protected zone decreased from 16.2 (±3.0) to 0% and from 32.3 (±5.1) to 2.5 (±1.6)% for *R. virginicus* and *R. flavipes*, respectively (Table 3). Note that the percentages of areas in Table 3 do not sum to the total for the whole box because the percentage values for each zone reflect the percentage excavation for that zone by itself. Significant decreases in excavated area in each zone because of dose ($\alpha = 0.05$, df = 3) were seen by ANOVA.

The model for the recovery of termites from the boxes was significant ($F = 6.64$; df = 7, 32; $P < 0.0001$), and significant effects because of dose ($F = 10.84$; df = 3; $P < 0.0001$) and species ($F = 8.80$; df = 1; $P = 0.0057$) were observed. The total number of termites recovered from the boxes was significantly lower at the highest dose, though a dose–response relationship at intermediate doses was not clear. In the control, 171 (±6.2) *R. virginicus* were recovered, and this declined to 83 (±27.8) at 500 ppm. For *R. flavipes*, 171 (±4.3) termites were recovered from the control, and 137 (±5.0) at 500 ppm. Fewer termites were recovered from the *R. virginicus* treatments at all doses except the control. Examination of the *R. virginicus* boxes revealed that in one box at the 250 ppm dose and three boxes at the 500 ppm dose, a red bacteria (probably *Serratia marcescens*) was present. The bacteria may have increased termite mortality, therefore reducing the average number of termites recovered. This outbreak was not observed in *R. flavipes*. Discarding these numbers, the average number recovered becomes 148 and 136 (out of 200) at the 250 and 500-ppm doses, respectively, which are still different from the control dose.

The number of termites recovered from the Barrier and Protected zones decreased with increasing dose, while the number of insects recovered from the Introduction zone increased relative to the control (Table 3). This is to be expected if the termites were avoiding the treated area. Excavation by *R. virginicus* into the Protected zone ceased at 250 and 500 ppm. Although excavation and termite recovery in the Barrier and Protected zones were reduced at 250 and 500 ppm in *R. flavipes*, termites nevertheless did penetrate and the term "barrier" is not appropriate.

In similar tests, *R. santonensis* did not penetrate a barrier of soil treated with ≈45,000 ppm isoborneol, but did penetrate some barriers treated with ≈32,000 ppm. In these cases they penetrated along the edge of the test boxes. Termites did not penetrate a circular barrier of ≈32,000 ppm isoborneol not bordering an edge of the box (Bläske and Hertel 2001). The amounts of catnip oil used in this study are roughly 4–20 times above that of imidacloprid, which is applied as a termiticide at ≈28 ppm (mg compound/kg soil, assuming a light mineral soil with a density of 2.6 g/ml). Fipronil is applied at ≈26 ppm.

In the coming years, natural products may play an important role in pest control, including control of termites. Information gathered now will determine the extent to which natural products will be successfully developed and used in the future. The results of the two barrier assays indicate that catnip oil acts as a barrier to termite tunneling. The effective doses tested were much lower than those reported for other monoterpenoids. These other monoterpenoids would be effective as barriers at lower doses as well if they had been tested. The longevity of the nepetalactone isomers is similar to other monoterpenoids, but less than other compounds used as soil treatments for termite control. The low toxicity of this oil when applied to the termite body surface indicates that the compound may be either poorly absorbed, or readily volatilized. In fumigation tests, the oil is roughly as toxic to the termites as other monoterpenoids. Volatility may contribute to a repellent effect by diffusing through the soil. The contact toxicity in the petri dishes is consistent with that of other monoterpenoids.

Nepetalactone as used in this study would be ineffective in controlling termites under field conditions. Overall, nepetalactone is not any more or less active than other monoterpenoids, but is less active than other compounds currently used for termite control. One issue hampering the oil's utility is persistence. Increasing its longevity in the soil environment after application may enhance the commercial potential of catnip essential oil or nepetalactone as a termite control product. Volatility may be lessened by formulation technology (such as encapsulation). Modification of the chemical structure could increase persistence and efficacy. Microbial degradation is another concern that must be investigated. Furthermore, the cost of catnip oil for use at effective rates is likely prohibitive, compared with other termiticidal compounds. Unless a way is found to competitively produce catnip oil and formulate it for long-term use, its use as a soil termiticide is limited. However, its use in other applications for termite control (such as fumigation for the control of isolated populations) may be feasible.

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