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ORIGINAL ARTICLE

Antitermitic effect of the *Lantana camara* plant on subterranean termites (Isoptera: Rhinotermitidae)

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Abstract The insecticidal effects of *Lantana camara* L. (flowers, leaves, stems and roots) and the soil where lantana had been growing, on foraging activity and survival of the subterranean termites *Coptotermes formosanus* and *Reticulitermes flavipes* were examined in a 3-week experiment. The soil in which lantana had been growing had no effect on termite tunneling and survival. Incorporation of chipped fresh lantana leaves and stems into soil had no effect on mortality but caused significant reduction in tunneling. The 5-cm wide barrier of soil with lantana tissue incorporated effectively repelled groups of both species from penetrating the barrier and thus prevented infestation of a piece of wood on the other side of the barrier. *C. formosanus* was more sensitive in avoiding the barrier than *R. flavipes*. Leaves, stems and flowers were more repellent than roots. These results provide preliminary evidence that fresh-cut lantana leaves, stems and flowers may have use as additives to garden mulches against termites.

Key words *Coptotermes formosanus*, *Lantana camara* L., mulch, nonchemical control, repellents, *Reticulitermes flavipes*, subterranean termites

Introduction

Since Wolcott's (1947) observations on the resistance of different wood species to termite attack, there have been numerous reports of wood extracts with insecticidal properties (reviewed by Scheffrahn, 1991). The environmental concerns over the persistent use of chemical termiticides have recently provoked interest in the search for plant-derived compounds (Cornelius *et al.*, 1997; Bläske & Hertel, 2001; Ganapaty *et al.*, 2004), as well as the use of live plants (Nix *et al.*, 2006) as alternatives to the use of synthetic insecticides in pest management programs. Although being a traditional cultural practice, the use of live plants is a minimum-risk and sustainable approach to termite management.

For the past 10 years, we have conducted numerous field trials evaluating new bait products or non-repellent termiticides for termite control. Both involve the use of stations for monitoring termite populations and control efficacy. The monitor stations are installed in-ground around houses, approximately 33 cm out from the wall/foundation and at 3-m intervals around the structures. Our records show that not all the monitor stations were visited by termites during the trial periods. A correlation and multiple regression analyses indicate that *Lantana camara* L. (Verbenaceae) near to the stations is among the environmental factors that may account for the absence of termites in monitoring stations that have never been foraged by termites, implying that *L. camara* might release compounds which inhibit termites from foraging within close vicinity to the plants.

L. camara is a common ornamental plant in the landscape in the southeastern US. It is native to subtropical and tropical America and has achieved worldwide

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distribution due to its promotion for ornamental cultivation in the early 19th century (Arora & Kohli, 1993; Ambika *et al.*, 2003). It is regarded both as a notorious weed in tropical regions and a popular ornamental garden and hedge plant for its beautiful multi-colored flowers in subtropical regions (Ghisalberti, 2000). It is also a versatile plant in that it is toxic to small ruminants feeding on it, but also has found various uses in folk medicine for treating many ailments (Sharma *et al.*, 1981). Lantana roots, leaves, stems and flowers contain several compounds with allelopathic, antimicrobial, nematicidal and insecticidal activities (Achhireddy & Singh, 1984; Begum *et al.*, 2000; Abdel-Hady *et al.*, 2005; Marongiu *et al.*, 2007; Sharma *et al.*, 2007). These compounds are mostly alkaloids including flavonoids, iridoid glycosides, sesquiterpenes, oligosaccharides and triterpenes (Ghisalberti, 2000).

One objective of our research efforts in preventive and remedial management of subterranean termites is to find alternatives to the broad-spectrum chemical biocides. The present laboratory study was to investigate whether or not the plant had repellent and toxic effects on subterranean termites. We determined the antitermitic repellency and/or toxic activities of lantana tissues, including flowers, leaves, stems and roots when lantana tissues were used as a green mulch barrier, as well as the soil in which the plants had been growing. Two economically important subterranean termite species were used for testing: the eastern subterranean termite, *Reticulitermes flavipes* (Kollar) and the Formosan subterranean termite, *Coptotermes formosanus* Shiraki.

Material and methods

Termites

Reticulitermes flavipes were collected from Auburn University campus and *C. formosanus* from the city of Opelika, Lee County, Alabama in June 2008. The collections were maintained in plastic boxes with moistened corrugated cardboard rolls as food at 24 ± 1°C and 80% ± 3% RH and were tested within 3 days of collection (Hu, 2005).

Plant and soil samples

L. camara var. *camara* (New Gold, yellow flowers) plants and soil in which the plants were growing were collected within 5 min of the bioassay. The plants, small bushes of 40 cm in height, were brought into the laboratory from one of the structures used in a bait trail on Auburn University campus. The roots were rinsed thoroughly with water to remove soil and debris. The fresh flowers, leaves, stems and roots were cut with scissors into approximately 0.5-cm long pieces. A volume of 10 × 20 × 30 cm (length × width × depth) soil was collected at 0.1–0.2, 0.5–0.6 and 1.0–1.1 m distances from the central root of a lantana plant. The soil collected at 10 m from the lantana plant in an open area served as control.

Barrier bioassays

The experiments were conducted in a dark incubator providing 24 ± 1°C and 80% ± 3% RH.

Experiment 1 was designed to examine the repellent and toxic effects of the soil in which the lantana plants had been growing. A 3 645 cm^3 Plexiglas container (27 × 15 × 9 cm) was used as the experimental arena. Four replicates were conducted for each distance (0.1–0.2, 0.5–0.6 and 1.0–1.1 m from the lantana plant central root) and the control. Each arena was prepared with a 2-cm deep layer of soil at the base, control soil at one side adjacent to the experimental soil on the other side, and two food substrates at opposite sides approximately 2 cm from the wall of the container (Fig. 1A). Each food substrate consisted of three pieces of southern yellow pine (2.0 × 0.5 × 0.5 cm) embedded in the soil. The entire soil layer (600 mL) was moistened with 180 mL of demineralized water. A group of 100 workers plus soldiers in their natural ratios (*R. flavipes*: 2 soldiers; *C. formosanus*: 10 soldiers) was introduced to the food substrate in the control soil by using a glass funnel. Experimental controls were run with only the soil taken 10 m from the lantana stand for the entire arena.

Experiment 2 was designed to determine the repellent and toxic effects of a barrier consisting of soil mixed with lantana tissue. The small pieces of flower, leaf, shoot and root tissues of lantana were incorporated into control soil at a ratio of 1 : 4 (w/w). The experimental arena was the same as aforementioned in experiment one. The treatment design was a modified version of Bläske and Hertel (2001) in that the 2-cm deep layer of control soil was partitioned in the middle by a 5-cm wide and 2-cm deep trench filled with 25% lantana mulch (Fig. 1B). Blank controls were run with unmulched soil in the entire arena. Four replicates were conducted for each mulch treatment and control.

Data were collected after 3 weeks. The bottom of each arena was visually examined to count the number of tunnels and the position of tunnels in response to each mulch barrier. For the qualitative analysis, the position of food substrates, test soil, and tunnels built by foraging termites, was copied on transparencies from the base of the

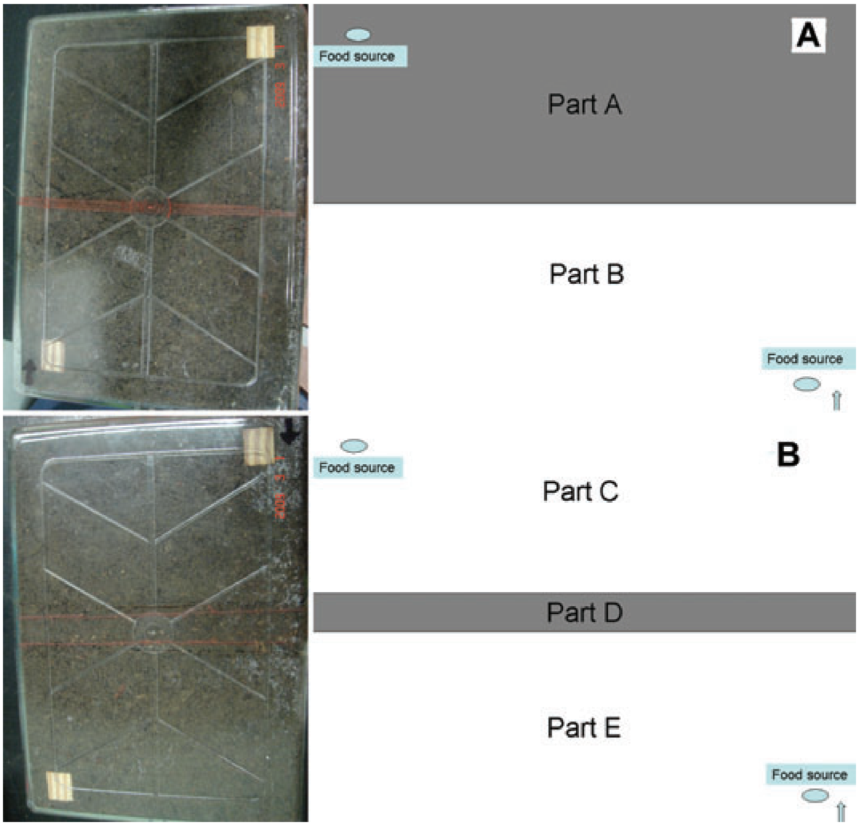

Fig. 1 (A) Choice test arena and barrier arrangement from experiment 1 for evaluating the response of subterranean termites to soil collected from where lantana plants had been growing. Part A (grey): soil collected from where the lantana plant had been growing; Part B (white): control soil. (B) Choice test arena and barrier arrangement from experiment 2 for evaluating the response of subterranean termites to soil with lantana plant tissue incorporated. Parts C and E: untreated soil (white); Part D (grey) soil treated with lantana tissue in the ratio 1 : 4 (w/w)) in the center of the arena: Southern Yellow Pine blocks in two locations. For further details see Materials and methods.

container. Subsequently, the soil and mulch were excavated and the number of surviving termites was recorded. Food substances were examined for termite damage. Percentage survival data were transformed by the arcsine of the square root, analyzed by a one-way analysis of variance (ANOVA) at $P \leq 0.05$, and compared by Tukey's honestly significant difference test (Statistix 9, 2008).

Results

Experiment 1

The soil collected from the field where lantana had been growing had no effect on termite foraging, survival, and the distribution of survivors (Table 1). Foraging termites tunneled through both the experimental and control areas in all replicates within the 3-week period and had moved into the second food substrate on the opposite side of the arena. There was no significant difference in the total tunnel lengths between *R. flavipes* and *C. formosanus* or between the soils collected at different distances from the central root of the lantana ($P > 0.05$). In all trials, surviving termites were distributed more or less evenly across the entire arena.

Experiment 2

In the tissue/soil mix treatments, termites built significantly fewer and shorter tunnels penetrating the barriers compared with controls (Fig. 2). Additionally, the barriers with flowers, leaves and shoots incorporated showed greater repellency to termite foragers than the barriers with roots.

The tissue/soil barriers had a significantly greater repellent effect on *C. formosanus* than on *R. flavipes*. *C. formosanus* built one short tunnel in only one of the four

Table 1 Tunnel length and survival (mean ± SD) of *R. flavipes* and *C. formosanus* and distribution of surviving termites after 3 weeks in a choice test with plain soil and soil collected at different distances from where the lantana was growing ($n = 4$ for each treatment).

Termite	Distance of soil sample from lantana plant	Total length of tunnel (cm)	Survival after 3 weeks (%)	Number of surviving termites in introduction site (part A) (%)
R. flavipes	Control	145.6 ± 11.2 a[†]	94.5 ± 3.7 a	47.7 ± 6.2 a
	0.1–0.2 m	167.4 ± 16.1 a	92.6 ± 5.2 a	56.7 ± 5.4 a
	0.5–0.6 m	152.5 ± 16.4 a	93.2 ± 4.8 a	52.4 ± 3.5 a
	1.0–1.1 m	157.8 ± 11.3 a	92.2 ± 5.5 a	39.5 ± 5.7 ab
C. formosanus	Control	127.2 ± 16.1 a	95.4 ± 1.2 a	49.3 ± 4.5 a
	0.1–0.2 m	144.6 ± 14.3 a	93.7 ± 2.5 a	56.6 ± 4.7 a
	0.5–0.6 m	136.5 ± 13.2 a	92.1 ± 4.6 a	52.4 ± 3.8 a
	1.0–1.1 m	133.2 ± 10.9 a	94.2 ± 3.8 a	47.2 ± 3.4 a

[†]Means followed by the same letter are not significantly different ($P < 0.05$, df = 3; ANOVA).

replicates of each treatment, passing the central barrier at the edge of the arena, but the termites did not approach the second food substrate on the protected side. For *R. flavipes*, one or two replicates in each treatment showed one to three tunnels passing the barriers, but feeding on the second food substrate occurred only in the flower and root treatments. In the controls, termites of both species tunneled throughout the entire arena of all the replications and infested both of the food sources at opposite locations.

The mean survival rates for all trials combined was 89.6% ± 3.0% ($n = 40$) in *R. flavipes* and 88.7% ± 3.5% in *C. formosanus* (Table 2). There was no significant difference in survival between control and treatments ($P > 0.05$, df = 3) or between the two termite species ($P > 0.05$). However, the distribution patterns of survivors were considerably different among the treatments and control. In controls and treatment of root/soil barriers, surviving termites distributed more or less evenly over the three parts of an experimental arena, indicating no repellent effect of lantana roots. In the barrier treatments with flowers, leaves or stems, a significantly large number of termites were found in introduction sites, a few in the areas past the barrier, and few (*R. flavipes*) or none (*C. formosanus*) inside the barriers. There were no dead termites observed in treated barriers.

Discussion

Termites readily tunneled through the soil of the entire arena with no significant difference between the treatments and control (Table 1), indicating that the soil

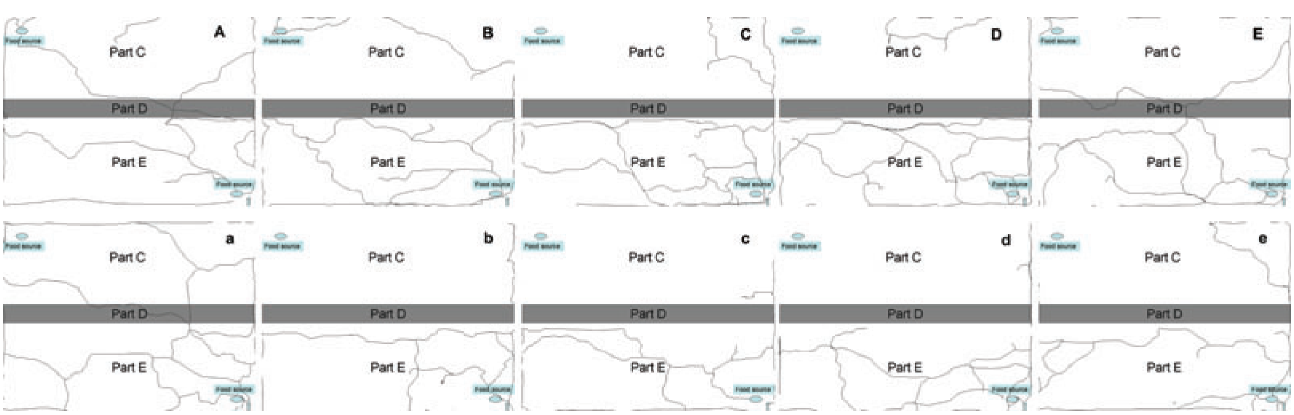

Fig. 2 Tunnels (A, B, C, D, E) built by *R. flavipes* and tunnels (a, b, c, d, e) built by *C. formosanus* within 3 weeks in test arenas with barriers (Part D) composed of control soil (A and a), 20% *L. camara* flower (B and b), 20% *L. camara* leaf (C and c), 20% *L. camara* stem (D and d) and 20% *L. camara* root (E and e).

Table 2 Survival of *R. flavipes* and *C. formosanus* and distribution of surviving termites after 3 weeks in arenas with a *L. camara* tissue-soil barrier.

	Treatment	Survival rate (%)†	Relative distribution of survivors in the test arena (%)		
			Introduction site (Part E)	Barrier (Part D)	Protected site (Part C)
R. flavipes	Control	93.2 ± 3.5 a	51.2 ± 4.7	11.2 ± 8.3	37.6 ± 7.7
	Flower-soil	89.6 ± 4.7 a	82.3 ± 10.7	1.7 ± 2.1	16.0 ± 5.7
	Stem-soil	88.8 ± 5.1 a	86.7 ± 9.3	2.2 ± 3.7	11.1 ± 5.3
	Leave-soil	85.2 ± 5.7 a	89.4 ± 8.3	0.7 ± 1.3	9.8 ± 6.7
	Root-soil	91.4 ± 3.3 a	46.4 ± 15.3	13.6 ± 5.3	39.8 ± 8.9
C. formosanus	Control	94.2 ± 2.8 a	46.9 ± 8.7	13.1 ± 5.2	40.1 ± 6.7
	Flower-soil	88.1 ± 3.7 a	92.2 ± 7.7	0	7.8 ± 8.3
	Stem-soil	86.2 ± 4.9 a	90.7 ± 6.3	0	9.3 ± 5.3
	Leave-soil	85.5 ± 4.6 a	95.7 ± 8.7	0	4.3 ± 3.7
	Root-soil	89.4 ± 3.6 a	52.3 ± 9.3	8.4 ± 7.7	39.3 ± 8.3

†Means followed by the same letter are not significantly different ($P < 0.05$, df = 3; ANOVA). Each mean ± SD is based on 400 termite workers (four replicates × 100 workers per replicate).

collected from where *L. camara* had been growing had neither repellent effect on termite foraging and the distribution of survivors, nor insecticidal effect on termites. A similar situation was reported by Achhireddy and Singh (1984) who found no allelopathic activity of lantana-associated soil in spite of a strong inhibitory effect of roots on seed germination and growth. Our results clearly indicate that the closeness of monitoring stations to lantana plants cannot explain why those stations were not visited by termites. Possible explanations for failing to link lantana presence with absence of termites are: (i) antitermitic volatiles are only associated with living plants and are short-lived in soil; (ii) the repellent effect is produced by aerial parts of the plant or originates from lantana leaf litter on the ground; (iii) the active compound(s) stay in the top soil, and do not move downward; or (iv) non-visitation of certain monitors was merely a coincidence. Laboratory assays with *R. hesperus* Banks by Smith and Rust (1991) provided evidence that toxic amounts of volatile insecticides do not move vertically nor horizontally from treated to untreated soil. Indirect exposure to untreated soil taken from beneath treated soil did not affect termite survival or behavior, whereas indirect exposure to treated soil increased termite mortality.

To elucidate potential insecticidal properties of natural extracts or live plants, it is useful to screen their efficacy in simple laboratory experiments (Cymorek & Pospischil, 1984; Singh & Mehta, 1998). The central soil barriers with different lantana tissue incorporated proved to be a satisfactory bioassay method. The central soil barriers with lantana tissue significantly prevented groups of *C. formosanus* (Fig. 2b–d) from penetrating the barrier and thus protected the second food substrate from termite attack. However, only the soil barriers with leaves and shoots were effective in preventing groups of *R. falvipes* from penetrating the treatment (Fig. 2C–D). Infestation of the food substrate on the protected side by *R. flavipes* via tunnels built along the edge of the arena occurred in one or two replicates of the flower and root treatments (Fig. 2B and E). This so-called edge effect is a common feature in the behavior of subterranean termites, and can often be seen in the field and in laboratory experiments (Forschler, 1998). In control situations, termite workers were distributed more or less evenly across the entire arena and showed no avoidance behavior.

The high survival rates of 89.6% ± 3.0% in *R. flavipes* and 88.7% ± 3.5% in *C. formosanus* (Table 2) and the fact that no dead termites were found in treated soil lead to the conclusion that *L. camara* tissues incorporated with soil in a choice situation, was not toxic but significantly repelled the two termite species. Our own laboratory experience attests that a survival rate of ≈ 90% within a month is normal for laboratory soil experiments with the two subterranean termite species (Hu, 2005).

Species-specific differences in the susceptibility of termites toward insecticide and repellents have been frequently reported (Petrowitz & Lenz, 1978; Su & Scheffrahn, 1990; Osbrink *et al.*, 2001; Hu, 2005). *C. formosanus* is the species usually more tolerant to chemical insecticides. However, in the present study, *C. formosanus* demonstrated a greater sensitivity and avoided significantly more the central barrier with lantana tissues added than *R. flavipes*.

For the past 50 years, termiticide-treated soil barriers have been conventionally used to exclude subterranean termites from buildings and structures in ground contact (Su & Scheffrahn, 1998; Lenz *et al.*, 1990; Forschler, 1994; Potter & Hillery, 2002; Hu & Hickman, 2006). The ban of organochlorine insecticides in the mid-1980s and recent phasing out of organophosphates due to environmental persistence and public health concerns, have revived interest in naturally occurring toxicants, semiochemicals and plants as alternatives to the current reliance upon insecticidal treatments (Bläske & Hertel, 2001; Ganapaty *et al.*, 2004; Nix *et al.*, 2006). More than 1 000 species of plants have been reported to have insecticidal properties, and a few have been developed for commercial use in pest control (Badshah *et al.*, 2005).

Natural products are often regarded as safe for humans by the US Environmental Protection Agency (EPA, 2000). However, the use of pure essential oils from toxic plants for pest control may not be an economically sound proposition, requiring not only more investigation of the cost–benefit relationship, but also their persistence under field conditions. Therefore, research on the evaluation of plants for termite control is necessary to help farmers and residents use locally available and eco-friendly products as a cheaper and sustainable alternative to insecticides. Our results provide preliminary empirical evidence that lantana plant materials (flowers, leaves and stems) may have use as an additive to garden mulches against termites. Our ongoing studies are investigating the persistent of the repellent performance of lantana tissue/mix against subterranean termites in both the laboratory and field trials.

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