Influence of Tree Extractives on Foraging Preferences of *Reticulitermes flavipes* (Isoptera: Rhinotermitidae)

by

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ABSTRACT

In addition to attacking buildings, *Reticulitermes flavipes* also forages on living trees in the northern portion of its geographic range in North America. An earlier survey of 17,800 boulevard and park trees in Toronto (Ontario, Canada) suggested that *R. flavipes* selectively attacked or avoided different tree species. Solvent extractions were performed of the bark, sapwood, and heartwood of a tree species frequently infested by *R. flavipes*, *Aesculus hippocastanum* (Hippocastanaceae), and of a tree very rarely infested, *Ailanthus altissima* (Simaroubaceae). When termites were forced to feed upon papers impregnated with 4% extractives, neither termite mortality nor relative amounts of feeding provided strong evidence of differential suitability of these two trees as hosts for *R. flavipes*. However, in behavioral assays, a majority of the *A. hippocastanum* extractives elicited positive termite orientation responses, while a majority of *A. altissima* extractives elicited negative responses. These results indicate that colonization of different tree species by *R. flavipes* can be correlated with the presence of behavioral chemicals eliciting either a positive or negative orientation response in the foraging termites.

INTRODUCTION

In North America, the eastern subterranean termite *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae) is found throughout the southeastern and northeastern United States, west along the southern shores of the Great Lakes, and as far north as the Town of Kincardine (44°11'N, 81°38'W) in the Canadian province of Ontario (Weesner 1970, Grace *et al.* 1989a, Grace 1990). This termite was apparently introduced to Toronto (43°42'N, 79°25'W) by ship from the United States between 1935 and 1938 (Urquhart 1953), and now infests buildings on at least 15% of the city blocks in metropolitan Toronto (Myles & Grace 1991).

In addition to attacking buildings in Toronto, *R. flavipes* also forages on living trees (Cooper 1984, Cooper & Grace 1987). This foraging behavior, in which *R. flavipes* constructs shelter tubes from the soil up

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the exterior surface of the tree bark, and excavates galleries between the inner and outer bark, is usually observed only in the northern portion of the termite's geographic distribution and is not common with other North American *Reticulitermes* species (Hagen 1885, Cooper & Grace 1987). *Reticulitermes flavipes* is selective in attacking trees, as is indicated by the results of an earlier survey of 17,800 boulevard and park trees, representing more than 20 species, in Toronto in which 4% were found to have termites present (Cooper & Grace 1987). Advanced age and rough bark texture appeared to contribute to termite attack (Myles & Grace 1991), as did the presence of decaying wounds (Cooper 1984). Exudates from such wounds are known to stimulate termite orientation (Esenther *et al.* 1961) due to compounds produced by the action of a number of different decay fungi (Watanabe & Cassida 1963, Smythe *et al.* 1967, Grace & Wilcox 1988). In fact, chemical factors are probably the most important determinants in termite foraging and food acceptance (Clément *et al.* 1988, Grace 1991).

The present study was initiated to determine whether a correlation existed between termite responses to tree chemistry (extractives) and the observed incidence of termite attack on different tree species in Toronto. Bioassays were designed to examine the relative importance of repellant and antifeedant effects in comparison to outright toxicity of such extractives. Studies with other *Reticulitermes* spp. have indicated that toxicity and repellency of wood extractives are not necessarily correlated (Grace *et al.* 1989b), and that extractives toxic in high concentration may even serve as positive orientation cues when present in low concentration (Clément *et al.* 1988). Based upon Cooper's (1981) survey, solvent extracts were prepared from the bark, sapwood and heartwood of a tree species frequently infested by *R. flavipes*, *Aesculus hippocastanum* L. (Hippocastanaceae), or horse chestnut (19.3% incidence of infestation); and from a tree very rarely infested, *Ailanthus altissima* (Mill.) Swingle (Simaroubaceae), or tree of heaven (0.7% incidence of infestation).

MATERIALS AND METHODS

Bark, sapwood and heartwood from freshly collected *A. hippocastanum* and *A. altissima* stemwood were separated and ground in a Wiley mill. The ground woods were then subjected to either a 12 hr continuous extraction in hexane or acetone in a soxhlet apparatus, a 24 hr continuous extraction in methanol in a soxhlet apparatus, or a 7 d cold soak (room temperature, ca. 22°C) in chloroform:acetone (1:1). Filtrates were concentrated by rotary evaporation and desiccation under vacuum in order to calculate percentage yields, then re-diluted in the appropriate solvent for application to filter paper. Extractive yields (Table 1) ranged from 0.3 to 10.2% (weight of dry extract / weight of wood).

Reticulitermes flavipes workers, externally undifferentiated individuals older than the third instar as determined by size, were collected using a trapping technique (Grace 1989a). Both a 15 d no-choice feeding assay and a short-term (20 min) orientation assay was performed with each solvent extract from each of the two tree species. In both assays, termite workers were exposed to filter papers (Whatman No. 3) saturated with the appropriate extractive dilution to achieve a standard 4% (weight of dry extract / weight of paper) extractive concentration in the paper.

In the no-choice feeding assay, 30 *R. flavipes* workers were placed in an open Petri dish on an air-dried filter paper disk containing 4% (weight/weight) extractives, and incubated in a dark cabinet at 27±0.5°C and 90±5% RH for 15 d. There were 3 replicates for each solvent extract, and control replicates in which the filter paper was treated with hexane:acetone:chloroform:methanol (1:1:1:1). At the end of the 15 d exposure, termite mortality was recorded and the filter papers oven-dried and weighed to measure mass loss due to feeding. Proportional mortality, transformed by the arcsine of the square root, and mass loss data were subjected to analysis of variance (ANOVA) and means significantly different at the 0.05 level were separated by the Ryan-Einot-Gabriel-Welsch multiple F test (SAS Institute 1987).

The orientation assay was performed with individual termites using the methods of Grace (1989a, 1989b). For each individual assay, 100 microliters of dilute extract was applied by pipette to a 23 mm filter paper disk to achieve a 4% extractive concentration. The disk was aired for 20 minutes to evaporate the solvent, then paired with a solvent-treated disk in a 5 cm diameter Petri dish. A single termite worker was placed in the center of the dish, between the two paper disks, and the position of the worker was recorded every 30 seconds for 20 minutes (a total of 40 observations). Fifty individuals were tested with each extract, using a new worker, new paper disks, and a clean dish for each assay. The proportion of the 50 individuals in contact with an extract-treated paper and the proportion in contact with a solvent-treated paper at each 30 second observation were compared over the 20 minute interval ($n = 40$ observations) with a paired comparisons *t* test (SAS Institute 1987).

RESULTS AND DISCUSSION

Extractive yields ranged from 0.6 to 10.2% for *A. hippocastanum*, and from 0.3 to 8.2% for *A. altissima* (Table 1). With both tree species, the greatest extractive yields were obtained from the bark. Only the hexane

Table 1. Percentage yield (wt. of extractives / wt. of wood) from wood extractions.

Tree Species	Solvent	Extractive Yield (wt/wt %) Bark	Sapwood	Heartwood
Aesculus hippocastanum	chlor:ace[a]	2.2	3.3	2.0
	methanol[b]	10.2	1.6	5.5
	acetone[c]	8.3	0.6	1.6
	hexane[c]	0.7	3.3	0.6
Ailanthus altissima	chlor:ace[a]	8.2	2.7	2.7
	methanol[b]	4.2	3.0	2.4
	acetone[c]	1.9	0.4	1.2
	hexane[c]	8.0	0.5	0.3

[a]7 day cold soak (room temperature, ca. 22°C) in chloroform:acetone (1:1).
[b]24 hour soxhlet extraction.
[c]12 hour soxhlet extraction.

Table 2. Mean (±SEM) percentage mortality of *R. flavipes* workers fed 15 days on filter papers containing 4% (wt/wt) wood extractives.*

Tree Species	Solvent	Source of Extractives Bark	Sapwood	Heartwood
A. hippocastanum	chlor:ace	21 ± 2ab	42 ± 12a	14 ± 4ab
	methanol	22 ± 8ab	12 ± 5b	10 ± 5b
	acetone	19 ± 3ab	18 ± 2ab	19 ± 3ab
	hexane	29 ± 6ab	17 ± 4ab	36 ± 1ab
	solvent control		23 ± 9ab	
A. altissima	chlor:ace	54 ± 19ab	50 ± 6ab	46 ± 9b
	methanol	44 ± 7b	37 ± 2b	31 ± 7b
	acetone	51 ± 10ab	41 ± 1b	62 ± 3ab
	hexane	51 ± 9ab	63 ± 9ab	90 ± 6a
	solvent control		39 ± 4b	

*N = 3 groups of 30 workers; SEM, standard error of the mean; means within each tree species followed by different letters are significantly different at the 0.05 level (ANOVA, REGW multiple F test).

extract of *A. altissima* heartwood caused significant termite mortality within 15 d (Table 2). However, the yield of hexane extractives from *A. altissima* heartwood was only 0.3%, while the test concentration on the filter paper was standardized at 4%. This suggests that less mortality would be expected from termite feeding on the natural wood substrate. Feeding, as measured by paper mass loss, was negatively correlated with termite mortality (Table 3), indicating that there was less feeding

Table 3. Mean (±SEM) paper consumption (mg) by *R. flavipes* workers fed 15 days on papers containing 4% (wt/wt) wood extractives.*

		Source of Extractives		
Tree Species	Solvent	Bark	Sapwood	Heartwood
A. hippocastanum	chlor:ace	12 ± 2abc	4 ± 3c	21 ± 2ab
	methanol	14 ± 3abc	21 ± 2a	17 ± 3abc
	acetone	13 ± 2abc	14 ± 3abc	18 ± 1abc
	hexane	11 ± 2abc	11 ± 1abc	10 ± 1bc
	solvent control		19 ± 7ab	
A. altissima	chlor:ace	18 ± 8ab	16 ± 2ab	18 ± 3ab
	methanol	22 ± 7ab	24 ± 3a	25 ± 3a
	acetone	17 ± 1ab	20 ± 2ab	15 ± 2ab
	hexane	18 ± 2ab	10 ± 2ab	7 ± 3b
	solvent control		22 ± 1ab	

*N = 3 groups of 30 workers; SEM, standard error of the mean; means within each tree species followed by different letters are significantly different at the 0.05 level (ANOVA, REGW multiple F test).

in those replicates where fewer termites survived. Thus, at least under the no-choice conditions of this feeding assay, no evidence of feeding deterrence was provided; nor does differential toxicity of the extractives in the two tree species alone serve to explain the pattern of termite attack observed under field conditions (Cooper 1981, Cooper & Grace 1987).

The orientation responses of individual *R. flavipes* workers to the tree extractives (Fig. 1) were more enlightening than the results of the no-choice feeding assays. Seven of the 12 *A. hippocastanum* extracts elicited significant ($P \leq 0.05$) positive orientation responses, and 3 elicited significant negative responses. The extractives eliciting positive responses represented 34.2% of the weight of the extracted bark, sapwood and heartwood matrices; while those eliciting negative responses represented 4.6% by weight. The opposite trend was observed with *A. altissima*, with 7 of the 12 solvent extracts eliciting negative orientation responses, and only 2 eliciting positive responses (Fig. 1). *Ailanthus altissima* extractives eliciting negative responses represented 24.8% by weight of the bark, sapwood and heartwood; while the 2 extractives eliciting positive responses represented 8.5%.

It is interesting to note that the more polar extractives, those soluble in methanol, elicited entirely positive termite orientation responses in the case of the frequently-infested *A. hippocastanum*, and entirely

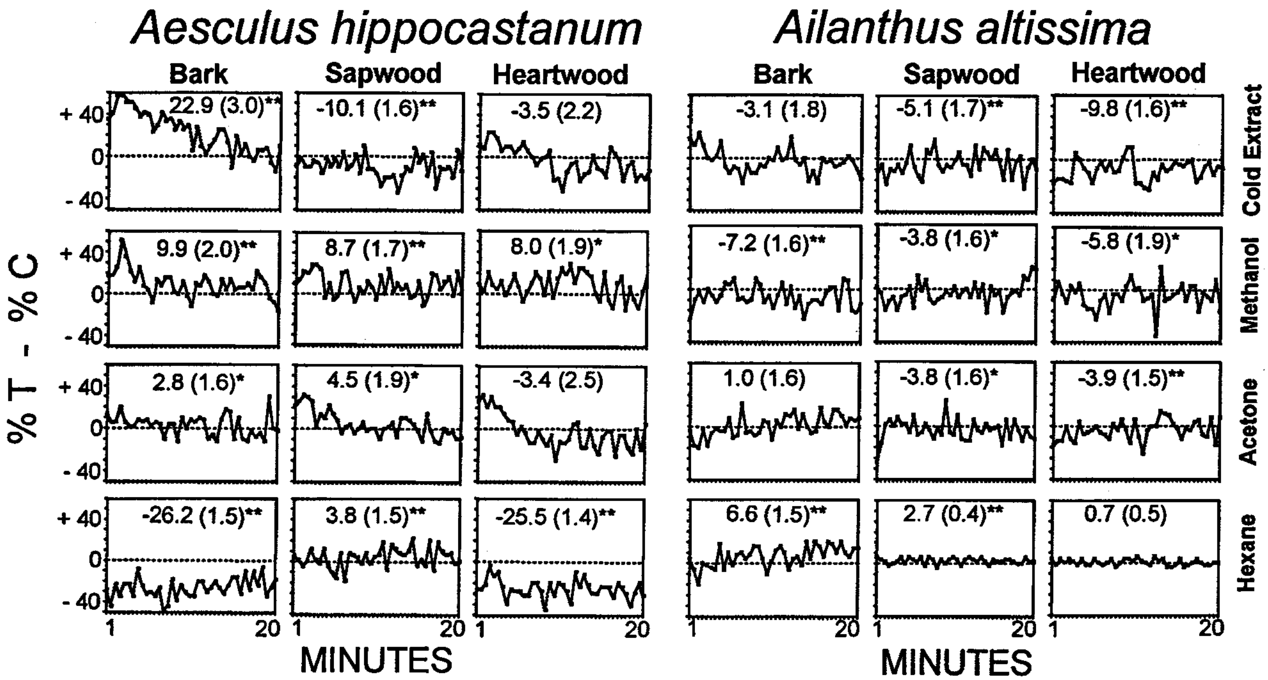

Fig. 1. Difference over a 20 minute period in the percentage of *R. flavipes* workers in contact with extractive-treated paper disks (%T) and the percentage in contact with solvent-treated papers (%C) in a behavioral assay. Each 30 second observation represents 50 individual assays, and numbers given are the mean (with standard error of the mean) percentage values over the entire period (n = 40 observations). A single asterisk indicates significance at the 0.05 level, and a double asterisk indicates significance at the 0.01 level (paired comparisons *t* test).

negative termite responses with the rarely-infested *A. altissima* (Fig. 1). Termites are able to detect chemical gradients, both on surfaces (Grace *et al.* 1988) and in the soil (Clément *et al.* 1988), and the more polar wood extractives are those most likely to be carried into the soil by precipitation or to be initially encountered by termites as they incorporate damp soil into galleries at the base of the tree or on the bark surface. Thus, the initial behavioral response of the leading foragers to these extractives may be the critical factor in either stimulating aggressive colonization of the tree or, conversely, retreat back into the soil.

These results underscore the critical role of behavioral chemicals (semiochemicals) in termite foraging, and the importance of including multiple-choice tests and behavioral observations in studies of termite feeding preferences, rather than relying solely upon observations of feeding and mortality in laboratory assays in which termites are presented with only a single test substrate as food. The present study provides a semiochemical-based explanation for an observed pattern of termite attack on living trees. However, the results of this study are equally applicable to termite location and preferential feeding upon fallen wood and other cellulosic materials, including those of potential utility as bait matrices for subterranean termite control around structures.

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