

TESTING ANTITERMITIC PROPERTIES OF BRAZILIAN WOODS AND THEIR EXTRACTS¹

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(Received April 1982)

ABSTRACT

A program on natural resistance of Brazilian woods to termites was initiated to obtain information valuable for more efficient utilization of various wood species of the Brazilian Amazon. Comparisons were made of the survival and feeding responses of *Reticulitermes flavipes* (Kollar) and *Coptotermes formosanus* Shiraki exposed to heartwood blocks and paper pads treated with extracts from eleven Brazilian woods. In no-choice block tests, no survival and little feeding occurred on *Calophyllum brasiliense*, *Carapa guianensis*, *Cedrela odorata*, *Diploptropis* spp., *Mezilaurus itauba*, *Ocotea cymbarum*, *Platymiscium ulei*, and *Sweetia nitens*. *Micrandra siphonioides* was the most favorable wood for both termite species. In choice block tests of all eleven woods, termites ignored most woods, eating primarily *M. siphonioides* and then small amounts of a few other woods.

Success in extracting antitermitic substances from milled heartwood of the eleven species varied with woods and solvents (successive cold extraction with hexane, acetone, 54:44:2 mixture of acetone-hexane-water, and 80% methanol). Termiticidal extracts were obtained from nine woods. In no-choice tests, *R. flavipes* did not survive the 8-week test period on seventeen extracts; *C. formosanus* did not survive on fourteen extracts. These extracts will be used for further study in research on potential termiticides from termite-resistant woods. Methods used in this study will be applied to tests in Brazil on various wood species against Brazilian termites.

Keywords: Termite control, *Calophyllum brasiliense*, *Carapa guianensis*, *Cedrela odorata*, *Diploptropis* spp., *Mezilaurus itauba*, *Ocotea cymbarum*, *Platymiscium ulei*, *Sweetia nitens*.

INTRODUCTION

Although the dense tropical forests of the Brazilian Amazon make up 30% of the world reserves of tropical wood, the region currently contributes only 3% of all tropical woods to the international market and about 10% to the internal Brazilian market (Van der Slooten et al. 1980). Contributing to the problems affecting the utilization of the Amazonian species are the great number of distinct species in highly heterogeneous forest stands, the questionable identity of many species, minor volume for certain species, and differences in physical appearance and properties of the species. Because so little is known about many Amazonian tree species (Brazier 1975), a concerted effort with the Brazilian Institute for Forestry Development (IBDF), Food and Agriculture Organization of the United

¹ The mention of a company or trade name is solely for identification and does not necessarily imply endorsement by the U.S. Department of Agriculture.

TABLE 1. *Brazilian woods in test for resistance to subterranean termites.*

Wood species	Common name	Family
<i>Calophyllum brasiliense</i> Camb.	Jacareuba	Guttiferae
<i>Carapa guianensis</i> Aubl.	Andiroba	Meliaceae
<i>Cedrela odorata</i> L.	Cedro	Meliaceae
<i>Copaifera multijuga</i> Hayne	Copaiba	Leguminosae
<i>Couropita subsessilis</i> Pilg.	Castanha de macaco	Lecythidaceae
<i>Diptotropis</i> spp.	Sucupira preta	Leguminosae
<i>Mezilaurus itauba</i> (Meissn.) Taubert ex Mez	Itauba	Lauraceae
<i>Micrandra siphonioides</i> Benth.	Ucuuba	Euphorbiaceae
<i>Ocotea cymbarum</i> H.B.K.	Louro inhamui	Lauraceae
<i>Platymiscium ulei</i> Harms	Macacauba	Leguminosae
<i>Sweetia nitens</i> (Vog.) Benth.	Itaubarana	Leguminosae

Nations (FAO), the National Amazonian Research Institute (INPA) in Manaus, and the Forest Products Laboratory in Brasilia is underway to determine the characteristics and properties of the various species and then to classify them for commercial and industrial uses (Van der Slooten et al. 1980). The overall objective is to make a more economic utilization of the heterogeneous forest of the Amazon region but in a rational and nonpredatory way. In addition to the workability characteristics and physical and mechanical properties of the woods, a critical factor for some uses is the woods' natural durability or how easily they can be treated with preservatives. Thus, knowledge of the woods' natural resistance to termites and the extractive constituents responsible for their resistance (Carter and Smythe 1974; Carter et al. 1975; Wolcott 1957) is important for more efficient utilization of the various wood species.

The primary purpose of this investigation was to initiate a program by the National Amazonian Research Institute in Manaus on natural resistance or susceptibility of Brazilian woods to deterioration by termites, thereby promoting more judicious use of the Amazonian species. The specific objectives were: to determine survival and feeding responses for two subterranean termites to heartwood blocks of eleven commercially important Brazilian hardwood species in both choice and no-choice tests, to identify solvent systems with which the antitermitic extractives can be removed, and to obtain extracts with antitermitic properties for mode of action studies and for sources for isolating and identifying the biologically active components. Methods developed by the U.S. Department of Agriculture, Forest Service, in Gulfport, MS, in their program on antitermitic properties to resistant woods were used.

MATERIALS AND METHODS

Except for minor changes, test materials were prepared and bioassayed as previously described for other wood species (Beal et al. 1974; Carter and Huffman 1982; Carter et al. 1979). For each of eleven tree species (Table 1), one heartwood board (ca. 15 × 60 × 2.5 cm) was obtained from a sawmill in Manaus, Brazil. Blocks (ca. 1.2 × 1.2 × 2.5 cm) were cut from each board and weighed. About one-half of the blocks were oven-dried at 103 C for 24 h and reweighed, and their moisture contents were calculated.

Shavings from each board were obtained with a jointer and then ground in a Wiley mill equipped with a 10-mesh screen. For each species, a portion of the milled heartwood was extracted successively with hexane (six times), acetone (ten times), a 54:44:2 acetone-hexane-water (AHW) mixture (six times), and 80% methanol (six times). Extraction of a wood with all four solvents was done during a 4-day period. After extraction with each solvent, a portion of air-dried extracted milled wood was set aside for testing against termites.

Separately for each wood, the filtrates for each solvent were combined, concentrated under reduced pressure in a rotary evaporator, and made up to known volumes. For conducting tests on the antitermitic properties of an extract, the volume of extract equivalent to that extracted from 0.5 g heartwood was added to an absorbent paper pad (ca. 0.5 g, 47 mm diameter, Gelman Instrument Co.), and the solvent was allowed to evaporate.

Termites

Two species of termites were studied: the eastern subterranean termite, *Reticulitermes flavipes* (Kollar), and the Formosan subterranean termite, *Coptotermes formosanus* Shiraki. As needed, externally undifferentiated termites beyond the third instar were taken from field-collected colonies and exposed to test materials. Test units were maintained at the optimal temperature for the termites: 25 ± 1 C for *R. flavipes* and 28 ± 1 C for *C. formosanus* (Carter and Huffman 1982).

No-choice tests

A test material (wood block, milled wood, or treated paper pad) was placed on moistened sand in a plastic container 5.0 cm diameter and 3.5 cm high, and 100 termites were added. Three replicates of both oven-dried (OD) and nonoven-dried (NOD) blocks for each wood species were established with each termite species. Tests were done on both NOD and OD blocks to determine if heating would volatilize or destroy antitermitic materials in the blocks. After 8 weeks, live termites were counted, and all wood blocks were oven-dried and weighed. The extent of termite feeding was estimated from the difference in the initial OD or calculated OD weight and the final OD weight of the block. Included in the tests for comparative purposes were controls of susceptible oven-dried slash pine sapwood blocks and starvation controls of sand substrate only.

In similar tests, unextracted and extracted milled heartwood (1.5 g for *R. flavipes* and 2.0 g for *C. formosanus*) and extract-treated paper pads (two pads equivalent to 1 g wood) were substituted for the test blocks. Untreated paper pads served as controls. Three replications of *C. formosanus* and two replications of *R. flavipes* were used with each treatment. Survival, but not feeding, was determined at 8 weeks.

Choice block tests

Eleven blocks, one of each wood species, were randomly placed in a plastic container 15 cm diameter by 6.3 cm high, containing 500 g sterile sand moistened with 70 ml distilled water (Carter and Smythe 1974). Three replicates of both OD and NOD blocks were used for each of the two termite species. To each test container, 500 termites were added. After 8 weeks, the surviving termites were counted and the blocks were oven-dried and weighed. Termite survival and block weight loss were determined.

TABLE 2. Mean weight loss of nonoven-dried (NOD) and oven-dried (OD) Brazilian wood blocks exposed in no-choice tests with termites.

Wood	<i>R. flavipes</i>				<i>C. formosanus</i>			
	NOD		OD		NOD		OD	
	%	mg	%	mg	%	mg	%	mg
<i>C. brasiliense</i>	1.1	21 a*	1.3	23 ab	2.2	38 ab	2.5	45 a
<i>C. guianensis</i>	0.9	22 a	1.1	27 b	2.9	73 abc	3.9	97 bc
<i>C. odorata</i>	2.0	37 ab	2.2	41 c	1.8	33 a	3.1	57 a
<i>C. multijuga</i>	3.3	71 c	2.3	51 d	9.4	204 d	7.3	162 d
<i>C. subsessilis</i>	3.8	51 b	3.9	54 d	8.5	114 c	7.7	106 c
<i>Diptotropis</i> spp.	1.1	34 ab	0.6	20 ab	2.9	88 bc	1.6	49 a
<i>M. itauba</i>	1.3	36 ab	0.9	25 ab	1.6	43 ab	1.3	36 a
<i>M. siphonioides</i>	7.3	136 d	15.0	281 e	18.0	338 e	19.4	362 e
<i>O. cymbarum</i>	0.9	23 a	1.0	24 ab	2.2	54 ab	2.6	67 ab
<i>P. ulei</i>	1.3	41 ab	0.8	23 ab	1.6	47 ab	1.7	51 a
<i>S. nitens</i>	0.7	23 a	0.5	16 a	1.2	38 ab	1.3	41 a
<i>Pinus</i> (control)	—	—	28.7	433	—	—	26.9	440

* Means (three replicates) in a column, followed by the same letter, are not significantly different at the 0.05 level of probability (Duncan's new multiple-range test).

Data analyses

The data were analyzed (Steel and Torrie 1980) by one-way analyses of variance ($P \leq 0.05$). For analysis, termite survival data were transformed to arc sine square root of percentage but are presented as untransformed percentage values in the tables to facilitate interpretation. Differences in termite survival and block weight loss were compared at the 0.05 level by Duncan's new multiple-range test. For each wood, differences in block weight losses between NOD and OD blocks or between *R. flavipes* and *C. formosanus* were compared by the *t*-test.

RESULTS AND DISCUSSION

No-choice block tests

Neither *R. flavipes* nor *C. formosanus* survived the 8-week test on most of the woods. *Micrandra siphonioides* Benth. was the most favorable wood to both termite species, but only 7% of *R. flavipes* survived the test on OD blocks and none survived on NOD blocks. With *C. formosanus*, 46% survived on NOD blocks and 67% on OD blocks of *M. siphonioides*. Low survival of the Formosan termite also occurred in one replicate each of NOD and OD blocks of *Copaifera multijuga* Hayne. No survival occurred on the other nine woods. In contrast, 78% *R. flavipes* and 83% *C. formosanus* survived on the susceptible slash pine sapwood control blocks. On the starvation controls of sand substrate only, all *R. flavipes* were dead by 3.5 weeks and *C. formosanus* by 7 weeks.

Although both species of termites consumed more than one-fourth of the susceptible slash pine control blocks, they caused little damage to most test blocks. Differences in block-weight loss means were tested with Duncan's new multiple-range test (Table 2). Greatest block-weight loss occurred on *M. siphonioides*, *C. multijuga*, and *Couropita subsessilis* Pilg.

The more aggressive *C. formosanus* generally ate more of all species than did *R. flavipes* (Table 2). Means for all eleven woods were: *C. formosanus*, 97 mg (NOD) and 98 mg (OD); *R. flavipes*, 45 mg (NOD) and 53 mg (OD). As a result

TABLE 3. Mean weight loss of nonoven-dried (NOD) and oven-dried (OD) Brazilian wood blocks exposed in choice tests with termites.

Wood	<i>R. flavipes</i> ^a				<i>C. formosanus</i> ^a			
	NOD		OD		NOD		OD	
	%	mg	%	mg	%	mg	%	mg
<i>C. brasiliense</i>	0.8	14 a*	0.8	15 a	2.2	41 a	2.0	34 ab
<i>C. guianensis</i>	0.6	15 a	0.8	21 a	0.9	23 a	1.1	27 a
<i>C. odorata</i>	1.3	24 ab	2.0	37 a	2.0	38 a	2.1	41 a
<i>C. multijuga</i>	2.5	55 b	2.5	56 a	3.9	86 a	5.5	119 c
<i>C. subsessilis</i>	1.1	16 a	1.4	20 a	6.1	81 a	8.2	109 bc
<i>Diptotropis</i> spp.	1.9	58 b	2.1	64 a	3.2	96 a	2.8	87 abc
<i>M. itauba</i>	0.8	22 ab	1.1	30 a	1.1	29 a	1.1	31 a
<i>M. siphonioides</i>	22.7	420 c	65.6	1,198 b	67.1	1,191 b	93.7	1,640 d
<i>O. cymbarum</i>	1.1	28 ab	1.3	33 a	2.3	57 a	2.6	64 abc
<i>P. ulei</i>	0.8	25 ab	1.1	33 a	1.4	40 a	1.0	32 a
<i>S. nitens</i>	0.4	13 a	0.7	22 a	0.8	25 a	0.6	19 a

^a Mean 8-week survival by *R. flavipes*: 0% on NOD blocks, 9% on OD blocks; by *C. formosanus*: 47% on NOD blocks, 78% on OD blocks.

* Means (three replicates) in a column, followed by the same letter, are not significantly different at the 0.05 level of probability (Duncan's new multiple-range test).

of heating the blocks, significant (*t*-test) improvement in feeding occurred only on *M. siphonioides* for *R. flavipes* and on *Carapa guianensis* Aubl. and *Cedrela odorata* L. for *C. formosanus*.

Choice block tests

In all choice tests, the termites ignored most of the woods, feeding mostly on the wood block of *M. siphonioides* and then lightly on a few other blocks (Table 3). The woods unfavorable for survival and feeding of the termites in the no-choice tests were also unfavorable in the choice tests. Very little, if any, wood was eaten from NOD or OD blocks of *C. guianensis*, *C. odorata*, *Mezilaurus itauba* (Meissn.) Taubert ex Mez, *Platymiscium ulei* Harms, and *Sweetia nitens* (Vog.) Benth. Because no block damage was observed visually, the small weight loss from these blocks probably resulted from leaching of water-soluble extrac-

TABLE 4. Mean survival of *R. flavipes* (*R.f.*) and *C. formosanus* (*C.f.*) on unextracted and extracted milled heartwood of three of eleven^a Brazilian wood species.

Wood species	Termite species	Survival on unextracted heartwood	Survival on extracted heartwood ^b			
			Hexane	Acetone	AHW	Methanol
-----%						
<i>C. multijuga</i>	<i>R.f.</i>	0	0	0	0	28
	<i>C.f.</i>	0	0	14	50	96
<i>P. ulei</i>	<i>R.f.</i>	0	0	0	0	0
	<i>C.f.</i>	0	0	0	0	9
<i>M. siphonioides</i>	<i>R.f.</i>	2	0	50	41	68
	<i>C.f.</i>	91	95	94	94	91

^a No survival on all test materials from *C. brasiliense*, *C. guianensis*, *C. odorata*, *C. subsessilis*, *Diptotropis* spp., *M. itauba*, *O. cymbarum*, and *S. nitens*.

^b Milled heartwood extracted successively with hexane, acetone, a 54:44:2 mixture of acetone-hexane-water (AHW), and 80% methanol.

TABLE 5. Survival of termites in no-choice tests for 8 weeks on paper pads treated with extracts^a from eleven Brazilian woods.

Wood	<i>R. flavipes</i>				<i>C. formosanus</i>			
	Hexane	Acetone	AHW ^b	Methanol	Hexane	Acetone	AHW	Methanol
	-----%-----				-----%-----			
<i>C. brasiliense</i>	82	0	20	78	82	0	0	64
<i>C. guianensis</i>	86	0	76	73	86	80	92	83
<i>C. odorata</i>	0	0	0	19	0	0	64	86
<i>C. multijuga</i>	80	0	54	0	88	0	88	14
<i>C. subsessilis</i>	80	79	78	80	85	85	85	88
<i>Diploptropis</i> spp.	83	0	0	0	78	0	0	74
<i>M. itauba</i>	57	67	0	0	83	84	67	0
<i>M. siphonioides</i>	75	54	77	80	81	80	82	88
<i>O. cymbarum</i>	74	0	68	78	86	0	81	81
<i>P. ulei</i>	78	0	0	65	71	0	0	0
<i>S. nitens</i>	70	0	0	51	73	0	0	9

^a For each test container, two paper pads (ca. 0.5 g each) were each treated with extracts equivalent to that extracted from 0.5 g heartwood.

^b Mixture of 54:44:2 acetone, hexane, and water, by volume.

tives into the wet sand. At the end of the 8-week tests with *C. formosanus*, the blocks of *M. itauba* in each of the six test containers had been completely covered with sand. Because termites had not fed on this wood, apparently they covered the blocks to avoid repellent or volatile antitermitic materials.

No *R. flavipes* survived in tests on NOD blocks and only 9% survived on OD blocks. Survival of *C. formosanus* averaged 47% on NOD blocks and 78% on OD blocks. Feeding corresponded with termite survival, with greater damage done on the OD blocks and by *C. formosanus*. Total consumption for all woods during the 8 weeks averaged 690 ± 61.4 mg on NOD blocks and $1,529 \pm 90.4$ on OD blocks by *R. flavipes* and $1,707 \pm 238.3$ mg on NOD blocks and $2,203 \pm 217.4$ mg on OD blocks by *C. formosanus* (Table 3). Major differences in overall wood consumption are reflected in the differences in consumption of NOD and OD blocks of *M. siphonioides* by the two termite species.

No-choice tests with milled wood and extracts

In general, the method of extracting antitermitic material(s) from the milled heartwood samples with a limited number of solvent extractions and no heat appeared to be inefficient. Some termites survived 8 weeks only on extracted samples of *M. siphonioides*, *C. multijuga*, and *P. ulei* (Table 4). In an earlier study with a group of North American hardwoods, similar extraction successfully removed the antitermitic material from many, although not all, of the woods (Carter 1979). Because extractive components of the various woods are chemically very diverse, no single sequence of extractions applies equally to all woods. However, the extraction method furnished antitermitic extracts from nine of the eleven Brazilian woods (Table 5).

In no-choice tests on absorbent paper pads treated with extracts from the eleven woods, termite survival varied with wood and solvent. Of the forty-four treatments (eleven woods and four solvents), no survival occurred on seventeen treatments (one hexane, eight acetone, five AHW, and three methanol extracts) with *R. flavipes* and on fourteen treatments (one hexane, seven acetone, four

AHW, and two methanol extracts) with *C. formosanus* (Table 5). Thus, acetone was overall the best of the four solvents for removing antitermitic material from these woods. With the exception of *C. odorata*, little termiticidal material was removed with the nonpolar solvent hexane. Paper pads treated with the hexane extract of *C. odorata* were very toxic to both termite species. For future work, hexane should be used to extract *C. odorata* but could be omitted for the other woods. A higher ratio of solvent to milled wood will be used in future extractions, and additional extractions will be made with the AHW mixture, 80% methanol, and warm distilled water.

When results are examined separately by wood species (Table 5), some termites survived in all tests with extracts of *C. subsessilis* and *M. siphonioides*, and *C. formosanus* also survived in all tests of *C. guianensis*. Survival was generally higher in tests with *C. formosanus* than with *R. flavipes*; more than 60% survival occurred with *R. flavipes* on twenty-one treatments and with *C. formosanus* on twenty-eight treatments.

CONCLUSIONS

Methods described in this paper for *R. flavipes* and *C. formosanus* will serve as a basis for tests in Brazil with these and other wood species against economically important Brazilian termites. Test results can vary greatly with different termite species (Becker 1969; Smythe and Carter 1970). A wood species resistant to one species of termites is not necessarily resistant to other species. Even in this study with *R. flavipes* and *C. formosanus*, both members of the family Rhinotermitidae, some differences in termite survival and feeding were apparent.

Testing for this study was essentially one of screening selected wood samples, using only one board for each of the eleven species (Table 1). Further testing on variability within a wood species will be done in Brazil with additional samples of the most resistant woods. Considerable variability in the amount of antitermitic material(s) present in wood can occur both within and between trees of the same species (Carter et al. 1979; Gay et al. 1955). Furthermore, these samples were from freshly cut trees, and the wood was not kiln-dried. Termites probably were killed in several tests from volatile antitermitic material(s). With drying or long-term storage, such antitermitic material may be depleted through evaporation. In tests with three species of *Eucalyptus*, Ruyooka (1978) found that blocks oven-dried at 105 C were more susceptible to termites than blocks vacuum dried at 21 C. However, in our tests little difference was noted with most of the wood species when both NOD and OD blocks were tested (Tables 2 and 3). Possibly, some materials were lost, but the original concentrations in the woods were so high that the remaining materials were sufficient to be effective against the termites. Greatest differences in OD and NOD wood were observed on blocks of *M. siphonioides*, the most favorable of the woods.

Currently, extracts possessing antitermitic properties against *R. flavipes* or *C. formosanus* are being tested for their mode of action on the termites. The extracts of some of these woods are not toxic but are repellent or distasteful to the termites, thereby resulting in termite mortality from starvation. The symbiotic protozoa, essential to the termites for digestion of cellulose, are affected by some of the wood extractives, either directly from protozoacidal activity or indirectly from induced starvation of the termite.

In future studies, biologically active fractions and isolated constituents will be obtained chromatographically from freshly prepared extracts of selected woods and then tested similarly for their antitermitic properties and mode of action. The identification and characterization of these antitermitic material(s) may lead to promising new and effective preservatives for susceptible wood. Either the biologically active constituents or synthesized compounds with similar structures might be used.

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