

# ANTITERMITIC AND ANTIFUNGAL PROPERTIES OF SELECTED BARK EXTRACTIVES<sup>1</sup>

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(Received February 1984)

## ABSTRACT

Screening trials were conducted to determine the antitermitic and antifungal properties of acetone:hexane:water (A:H:W) bark extracts obtained from five northeastern trees: *Pinus resinosa*, *P. strobus*, *Carya ovata* Mill., *Quercus rubra*, and *Acer rubrum*. Extract-treated (0.07 g/ml) cellulose pads were used to evaluate antitermitic properties, while extract-treated (0.01 and 0.1 g/ml) glucose-asparagine growth media inoculated with *Lenzites trabea* were used to evaluate antifungal properties.

Complete termite mortality occurred on cellulose pads treated with the extracts from *C. ovata* and *Q. rubra*. Near complete mortality was observed with the *P. strobus* extracts. Significant antifungal effects were observed on the growth media treated with bark extracts of *C. ovata*, *Q. rubra*, and *P. strobus*. Flasks treated with extracts at the 0.1 g/ml levels exhibited the greater effect.

*Keywords:* Bark extractives, termites, fungi, *Carya ovata*, *Quercus rubra*, *Acer rubrum*, *Pinus resinosa*, *Pinus strobus*.

## INTRODUCTION

Certain wood species are naturally resistant to termite attack and decay organisms, and the durability of wood is attributed to the amounts and types of extractives found in the heartwood (Rudman and DaCosta 1959; Rudman et al. 1967; Saeiki et al. 1971; Ocloo 1978). Rudman (1959, 1965, 1967), in his studies, found callitric acid to be both termite-repellent and toxic to fungi. He also reported that a group of neutral extractives, namely cudesmol and azulene, are important in the decay resistance of cypress pine. Stilbene, a pinosylvin monoethyl ether compound, has also been isolated from pine heartwood and appears to be both fungistatic and fungitoxic (Shain 1967; Hillis and Inoue 1967; Coutts 1970).

Tannins resist fungal growth, and are another group of extractives found both in wood and bark. Cruickshank and Perrin (1964), Anderson (1961) and Rudman (1965) reported that tannins inhibit growth of many fungi in culture. Materials extracted from white oak heartwood with hot water were highly toxic to *Lenzites trabea* grown in a malt extract agar culture.

Earlier studies showed that the most successful method of removing these extractive materials from the wood appeared to be with a 54:44:2 mixture of acetone:hexane:water (A:H:W) (Carter and Smythe 1974; Nelson 1975; Carter et al. 1979; Carter and Huffman 1982). Most recently, Steller (1982) reported on the antitermitic properties of selected bark extractives and found termite mortality to occur with A:H:W bark extracts obtained from the species *Quercus prinus*, *Pinus strobus*, *Carya ovata* Mill., and *Sassafras albidum*.

Since the tissues comprising the heartwood and bark originate from the same

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<sup>1</sup> This paper has been approved as Journal Series No. 6868.

secondary meristem, one might expect to find almost similar types of compounds from both tissues. Therefore, screening trials were conducted to evaluate the antitermitic and antifungal properties of A:H:W bark extracts from five north-eastern trees. Tests were conducted using cellulose paper pads treated with A:H:W bark extracts for antitermitic trials and glucose-asparagine growth mediums inoculated with a brown rot fungus and with added A:H:W bark extracts for antifungal trials.

#### EXPERIMENTAL

##### *Bark selection and preparation*

Five species were selected for study—two softwoods and three hardwoods. The species selected were red pine (*Pinus resinosa*), white pine (*P. strobus*), shagbark hickory (*Carya ovata*), red oak (*Quercus rubra*), and red maple (*Acer rubrum*). Both shagbark hickory and white pine bark were selected because of antitermitic properties observed in earlier studies. Bark (rhytidome) was hewed from three randomly selected trees, air-dried, and each sample was Wiley milled to pass a 40-mesh screen. A total of 15 bark samples were prepared (5 species  $\times$  3 trees) and stored in paper bags.

Acetone:hexane:water (54:44:2 by volume) extraction procedures were followed according to the methods described by Steller (1982). Briefly, four 20-g bark samples were extracted in a Soxhlet extractor, combined, filtered, and stripped of solvent in a rotary vacuum evaporator at 30 C. Sufficient solvent was removed to obtain the desired concentration level (0.07, 0.1, and 0.01 g/ml) for treatment of cellulose paper pads or the preparation of bark extractive treated glucose-asparagine mixtures.

##### *Antitermitic trials*

The eastern subterranean termite, *Reculitermes flavipes* (Kollar) was used, and antitermitic trials were conducted according to methods described by Steller (1982) with but one exception. Cellulose paper pads were treated at 0.07 g/ml (bark extracts) treatment level as opposed to the higher treatment levels used in earlier studies.

A:H:W bark extracts obtained from each tree and species were used to treat cellulose paper pads and were tested in triplicate in a circular planter petri dish (5.08  $\times$  1.27 cm). Fifty *R. flavipes* workers were added to the pads and the 4-week trial was conducted in an environmental chamber at 21 C and 50% relative humidity. To determine the effect of solvent (A:H:W) on termite survival, paper pads were treated with and without the solvent for controls. Two separate trials were performed for each extraction with controls for a total number of 126 observations.

##### *Antifungal trials*

The brown-rot fungus *Lenzites trabea* belonging to the Basidiomycetes was used for the antifungal trials. Brown-rot fungi generally utilize only the holocellulose components of wood while leaving lignin almost unaltered. This fungus normally causes decay of wood exposed above the ground.

*Lenzites trabea* was initially cultured on malt-agar (3%) prior to the fungistatic

testing. Glucose-asparagine medium was prepared according to the methods described by Lilly and Barnett (1951). Approximately 3 mm (in diameter) of mycelium and agar was cut from the edge of the fungus colony and was transferred to a 250 ml flask containing 15 ml of aqueous glucose-asparagine medium. The A:H:W bark extracts from each bark species were added to the flasks at two concentration levels of 0.01 g/ml and 0.1 g/ml. Extract concentration was determined on the weight of the residue remaining after the solvent was removed with a rotary flash evaporator. An A:H:W solvent control was also prepared and tested to determine what effect the solvent had on fungus growth. In addition, a glucose-asparagine solution was prepared and inoculated with the fungi as a control. All glassware was autoclaved, and aluminum foil was used to cover the mouth of each flask to minimize the risk of contamination. The inoculated flasks were incubated at 26 C for 14 days, and the mycelium was collected by filtration onto a tared filter paper. The collected mycelium was washed with distilled water and dried to a constant weight at 80 C and weighed.

Visual observations were also made during testing to assess the amount of growth of mycelium in the flasks. A subjective test was performed, whereby the amount of growth of the fungus contained within a flask was designated by the following numbering system. They were: 5 = very high growth, 4 = high growth, 3 = medium growth, 2 = low growth, 1 = sparse, 0 = very sparse, and NG = no growth.

During the fungistatic trials, it was observed that the harvested mycelium contained a waxlike residue that was trapped on the filter paper. This waxlike material was believed to be suberin, which is a component of bark. Earlier chemical analysis studies on these barks showed that suberin content can vary both within and among species (Harun 1983). The suberin content determined for each tree bark was therefore subtracted from the final weight of the test flask and this corrected value was reported in this study.

#### *Data analysis*

The data were analyzed using the least squares analysis of variance. Differences in means within and among species were determined using Duncan's new multiple range test (Steel and Torrie 1960).

### RESULTS AND DISCUSSION

#### *Antitermitic trials*

Both within species and among species evaluations of the antitermitic properties of A:H:W bark extracts are summarized in Tables 1 and 2. No within species differences in termite feeding (pad weight loss) and termite survival were observed in Trial 1 (Table 1). However, within species differences in termite survival were observed in Trial 2 for the A:H:W bark extracts obtained from red pine and white pine. Differences in termite survival were also observed among the controls indicating that possibly slight differences in test conditions occurred between trials. With but one exception in Trial 2 (white pine, tree 2), the A:H:W bark extracts from the species shagbark hickory, red oak, and white pine exhibited complete termite mortality after the 4-week trial period.

Comparisons in termite survival among species examined and the controls

TABLE 1. Within species feeding (pads weight loss) and survival of *R. flavipes* exposed to cellulose pads treated with A:H:W<sup>1</sup> bark extractives at 0.07 g/ml treatment level.

Species	Tree	Trial 1		Trial 2		Average of Trial 1 and 2	
		Termite survival	Pad weight loss	Termite survival	Pad weight loss	Termite survival	Pad weight loss
		%					
Red pine	1	14.0 <sup>2</sup>	13.6	15.3a <sup>2</sup>	17.1	14.7a	15.1
	2	10.0	13.3	6.7c	5.0	8.3ab	9.1
	3	8.7	9.1	14.7b	11.2	11.7b	12.3
White pine	1	0	5.3	0 b	1.1	0 b	3.2
	2	0	10.0	6.7a	9.4	3.3a	9.7
	3	0	5.4	0 b	8.6	0 b	7.0
Shagbark hickory	1	0	1.9	0	7.2	0	4.6
	2	0	1.7	0	9.1	0	5.4
	3	0	0.6	0	6.5	0	3.6
Red oak	1	0	0.9	0	6.1	0	7.6b
	2	0	5.8	0	4.6	0	5.3b
	3	0	15.0	0	26.0	0	20.5a
Red maple	1	17.3	16.4	11.3	15.4	14.3	15.9
	2	18.0	9.1	14.7	17.0	16.3	13.9
	3	18.0	12.0	14.0	17.8	16.0	16.0
Control	—	72.3	17.2	86.7a	13.1	79.7	15.1
	—	70.0	17.4	74.0b	12.6	72.0	15.0
	—	56.7	16.1	78.0ab	13.7	68.3	14.9
A:H:W	—	56.7	18.0	78.0	12.0b	67.3	15.0
	—	58.7	15.9	62.7	18.8a	60.7	17.4
	—	57.7	21.9	84.7	14.1ab	74.7	18.0

<sup>1</sup> A:H:W, Acetone:hexane:water, 54:22:2 by volume.

<sup>2</sup> Each measurement in Trials 1 and 2 is an average of 3 replicates. A small letter (a, b, c) within a column and species indicate that a significant difference occurred at the 0.05 level of probability (Duncan's new multiple-range test).

showed that all the A:H:W bark extracts examined exhibited termite mortality (Table 2). For both trials, A:H:W extracts from shagbark hickory and red oak caused complete mortality, whereas white pine bark extracts exhibited an average survival rate (Trial 1 and 2) of less than 2% after the 4-week trial period (Table 2).

Pad weight loss (percent) due to termite feeding on cellulose pads treated with A:H:W bark extracts was also examined. Results showed that the A:H:W bark extracts that exhibited the highest termite mortality also exhibited the lowest pad weight loss. The average weight loss (Trial 1 and 2) for A:H:W bark extract treated pads ranged from a low of 4.7% for shagbark hickory to a high of 16.8% for the A:H:W control (Table 2). Pad weight loss was significantly lower for those pads treated with the A:H:W bark extracts from the species shagbark hickory and white pine compared to the other barks examined (Table 2).

Results observed in this study support earlier findings reported by Steller (1982) in that A:H:W bark extracts from shagbark hickory and white pine inhibit termite activity. However, in this study complete termite mortality was observed to occur for paper pads treated with A:H:W bark extracts at the 0.07 g/ml treatment level for shagbark hickory and red oak. This was not observed in earlier work in that

TABLE 2. Among species feeding (pad weight loss) and survival of *R. flavipes* exposed to cellulose pads treated with A:H:W<sup>1</sup> bark extractives at 0.07 g/ml treatment level.

Species	Trial 1		Trial 2		Average of Trials 1 and 2	
	Termite survival (%)	Pad weight loss (%)	Termite survival (%)	Pad weight loss (%)	Termite survival (%)	Pad weight loss (%)
Red pine	10.8 <sup>2</sup> d	12.0ab	12.2b	11.1abc	11.5 <sup>3</sup> c	11.5ab
White pine	0.0e	6.9bc	2.2c	6.3c	1.1d	6.6c
Shagbark hickory	0.0e	1.4c	0.0c	7.6bc	0.0d	4.7c
Red oak	0.0e	9.9b	0.0c	12.3abc	0.0d	11.1ab
Red maple	17.7c	12.5ab	13.3b	16.7a	15.5c	14.6a
Control	67.1a	16.9a	79.5a	13.1abc	73.3a	15.0a
A:H:W	60.1b	18.6a	75.2a	15.0ab	67.6b	16.8a

<sup>1</sup> A:H:W. Acetone:hexane:water, 54:44:2 by volume.

<sup>2</sup> Each figure is an average of 9 replicates.

<sup>3</sup> Each figure is an average of 18 replicates.

Means in the same column with the same letter are not significantly different at 0.05 level of probability (Duncan's new multiple-range test).

termite survival occurred at the 0.16 g/ml treatment level. Since different tree barks were extracted, it is possible that within species variations in A:H:W extractives occurred and could account for the differences observed between the two studies. Nevertheless, the results do show that the A:H:W bark extract from shagbark hickory and red oak were antitermitic and inhibit termite activity.

#### Antifungal trials

The A:H:W bark extracts from all species evaluated had a positive effect in reducing the fungal growth of *L. trabea* compared to the control samples (Tables 3 and 4). Within species differences in antifungal properties were measured for

TABLE 3. Within species growth (oven-dry weight in mg) of *L. trabea* in glucose-asparagine growing media treated with A:H:W<sup>1</sup> bark extractives at 0.1 g/ml and 0.01 g/ml treatment levels.

Species	Treatment level	Fungi growth (mg)		
		Tree 1	Tree 2	Tree 3
Red pine	0.1 g/ml	1.30	1.40	1.30
	0.01 g/ml	3.90AB <sup>2</sup>	3.40B	4.90A
White pine	0.1 g/ml	0.0	0.41	0.04
	0.01 g/ml	1.21	1.00	1.62
Shagbark hickory	0.1 g/ml	0.20	0.20	0.54
	0.01 g/ml	0.95	0.95	1.08
Red oak	0.1 g/ml	0.09	0.24	0.54
	0.01 g/ml	2.22A	0.64B	2.08A
Red maple	0.1 g/ml	1.63	2.73	1.41
	0.01 g/ml	4.66	4.51	3.86
Control <sup>3</sup>	—	9.89	10.10	10.46
A:H:W <sup>4</sup>	—	6.79	5.54	7.75

<sup>1</sup> Acetone:hexane:water, 54:44:2 by volume.

<sup>2</sup> Each measurement is an average of 3 replicates. A capital letter (A, B, C) within a row and species indicates that a significant difference occurred at the 0.05 level of probability (Duncan's new multiple-range test).

<sup>3</sup> Glucose-asparagine solution.

<sup>4</sup> Acetone:hexane:water, 5% in vol./vol. ratio with glucose-asparagine solution (growing media).

TABLE 4. Among species growth (oven dry weight in mg) of *L. trabea* in glucose-asparagine treated with A:H:W<sup>1</sup> bark extractives at 0.1 g/ml and 0.01 g/ml treatment levels.

Species	Fungi growth (mg) at 0.1 g/ml treatment level	Fungi growth (mg) at 0.01 g/ml treatment level
Red pine	1.35cd <sup>2</sup>	4.08c
White pine	0.15d	1.28d
Shagbark hickory	0.07d	1.19d
Red oak	0.29d	1.65d
Red maple	1.92c	4.34c
Control <sup>3</sup>	10.15a	10.15a
A:H:W <sup>4</sup>	6.69b	6.69b

<sup>1</sup> A:H:W, Acetone:hexane:water, 54:44:2 by volume.

<sup>2</sup> Each measurement is an average of 9 replicates. Means in the same column with the same letter are not significantly different at 0.05 level of probability (Duncan's new multiple-range test).

<sup>3</sup> Glucose-asparagine solution.

<sup>4</sup> Acetone:hexane:water, 5% in vol./vol. ratio with glucose-asparagine solution.

red pine (tree 1) and red oak (tree 2) at the 0.01 g/ml treatment level; however, no within species differences were measured at the 0.1 g/ml treatment level (Table 3). As expected, the 0.1 g/ml treatment level produced less mycelial growth than did the 0.01 g/ml treatment level for all barks tested.

Comparisons among the A:H:W bark extracts examined showed that the extracts obtained from shagbark hickory, red oak, and white pine exhibited significantly lower mycelial growth than did the species red maple and red pine (Table 4). These results parallel earlier observations in that high termite mortality occurred on cellulose pads treated with these bark extracts. Visual assessments on the fungal growth tend to support the quantitative measurements observed in this study (Table 5). Mycelium growth appeared to be lower for those treated flasks that contained the white pine, shagbark hickory, and red oak bark extract, whereas the treated flasks that contained red pine and red maple bark extracts and the controls exhibited high mycelium growth as indicated by the visual ranking system employed in this study.

The effectiveness of bark extracts in retarding both termite activity and fungal growth may be dependent not only on the toxic elements contained in the bark extracts, but also on the extractive concentration. Zabel (1948) and Rudman (1962) postulated that the durability of oak heartwood was attributed to the toxicity of tannins as well as to their high concentration in the wood. Zabel (1948) indicated that the amount and toxicity of the tannins in oak heartwood decrease radially from the outer heartwood (region of higher concentration) towards the pith (region of lower concentration). Rudman (1962) found that the nondurable heartwood of *Eucalypts regnans* contained the same toxic components (tannins) as did the durable heartwood of *E. microcorys* and *E. triantha*; however, the concentration was much higher in the latter two species.

#### Chemical considerations

Oak bark contains the chemical components D-catechin, D-galocatechin, leucopelargonidin, leucocyanidin, leucodelphinidin, gallic acid and various condensed tannins based on catechin-galocatechin polymers (Rowe and Conner 1979). The presence of these compounds in A:H:W bark extracts might explain the antitermitic and antifungal properties of northern red oak bark. Tannins present

TABLE 5. Visual assessment of growth of *L. trabea* in glucose-asparagine treated with A:H:W<sup>1</sup> bark extractives at 0.1 g/ml and 0.01 g/ml treatment levels.

Tree	Replicates	Trial	Species				Control <sup>1</sup>	A:H:W <sup>4</sup>	
			Red pine <sup>2</sup>	White pine	Shagbark hickory	Red oak			Red maple
1	a	1	0	NG	NG	NG	2	5	3
		2	2	1	1	2	3	—	—
	b	1	1	NG	0	NG	1	5	4
		2	3	1	2	2	3	—	—
	c	1	1	NG	NG	0	2	4	5
		2	3	2	1	2	3	—	—
2	a	1	0	NG	NG	NG	2	4	4
		2	2	1	1	0	3	—	—
	b	1	1	0	0	NG	1	5	4
		2	3	1	1	1	3	—	—
	c	1	2	NG	0	1	2	5	4
		2	2	1	1	1	3	—	—
3	a	1	0	NG	NG	0	1	4	5
		2	3	2	1	2	3	—	—
	b	1	2	NG	NG	1	1	5	3
		2	3	2	2	2	2	—	—
	c	1	1	0	NG	0	2	4	4
		2	3	1	2	1	3	—	—

<sup>1</sup> Acetone : hexane : water, 54:44:2 by volume.

<sup>2</sup> Visual assessment of fungi growth: 5—very high growth; 4—high growth; 3—medium growth; 2—low growth; 1—sparse; 0—very sparse; NG—no growth.

<sup>3</sup> Glucose-asparagine solution.

<sup>4</sup> Acetone : hexane : water, 5 percent vol./vol. ratio with glucose-asparagine solution.

in the heartwood of oak are known to enhance wood durability. Saeiki et al. (1971) found that traces of ellagitannins in northern red oak heartwood and its occurrence could be the contributing factor in inhibiting fungi growth as Hart and Hillis (1974) observed in their study. The occurrence of any of these chemicals may have contributed to the preservative properties of A:H:W bark extracts.

According to Rowe and Conner (1979), red maple bark contains the following components: tannins, glucose,  $\beta$ -sitosterol, D-catechin, pyrocyanidin, pyrogallol, catechin and gallic acid. Gallic acid and catechin are known antifungal compounds produced by the tree. These compounds may also have antitermitic properties. Although the red maple bark extracts (A:H:W) showed the least antitermitic and antifungal properties compared to the other four species of bark extract examined, they did exhibit some degree of toxicity.

Juglone and other naphthaquinone type compounds can be isolated from shagbark hickory bark extracts. Since beetles are known to be deterred from feeding on the bark of shagbark hickory, it is speculated that these types of compounds may account for the antitermitic and antifungal properties observed in this study. In both antitermitic and fungistatic trials, shagbark hickory A:H:W bark extracts exhibited the highest termite mortality and least amount of fungi growth.

Pine barks are generally known to have high tannin contents. Rudman (1965) and Rowe and Conner (1979) reported that these tanninlike compounds are detrimental to termites. Besides tannins, pine bark also contain phenolic compounds, which may further contribute to the termiticidal and fungistatic properties of the bark. In this study, red pine bark extracts appeared to be less effective in termi-

ticidal and fungistatic properties compared to white pine bark extracts. A possible explanation may be in the toxicity and the amount of detrimental compounds present in both bark extracts.

#### SUMMARY AND CONCLUSIONS

On the basis of observations made in this study, the following conclusions can be drawn:

1. When compared with termite survival in controls, termite mortality occurred on cellulose paper pads treated with the A:H:W bark extracts from the species shagbark hickory, red oak, white pine, red pine and red maple; complete termite mortality occurred with the bark extracts from shagbark hickory and red oak after the 4-week trial period.
2. Glucose-asparagine solutions inoculated with *L. trabea* and treated with A:H:W bark extracts exhibited less fungal growth than did the controls; however, only shagbark hickory, red oak, and white pine bark extracts exhibited significantly lower fungal growth compared to the other barks examined.
3. Less mycelial growth was harvested from flasks treated with A:H:W bark extracts at the 0.1 g/ml compared to flasks treated with A:H:W bark extracts at the 0.01 g/ml treatment level.
4. A:H:W bark extract from shagbark hickory, red oak, and white pine inhibit termite activity and fungi growth of *L. trabea*.

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