Methods for Evaluating Fungal Inhibition and Barrier Action of Tree Wound Paints

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ABSTRACT

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Six commercial asphalt tree wound paints were evaluated in vitro for fungal inhibition and mechanical barrier action to *Lenzites trabea, Stereum frustulatum, Poria placenta,* and *Coriolus versicolor.* The paints alone were not fungitoxic and did not provide a mechanical barrier. Paints containing 3.3 and 10% copper naphthenate were inhibitory and provided an inhibitive barrier against the fungi. The test methods may be valuable for evaluating potential tree wound paints.

Tree wound paints registered by the Environmental Protection Agency are claimed to have fungicidal activity against wood decay fungi, but evaluation of their efficacy in field tests has been difficult. Young and Tilford (14) found that asphalt paints promote rapid tree wound healing and are superior to other materials in preventing decay in oak, poplar, and red maple. Tilford (11) reported a decay fungus growing abundantly on both liquid asphaltum dressings and water-asphaltum emulsions. He painted filter paper with the test compound and, after it dried, applied a sugar solution. The treated filter paper was then inoculated with *Fomes annosus.*

Walter and Mook (12) evaluated asphalt wound dressings contaminated with *Ceratostomella*-infected sawdust and found that the fungus could survive in the dressings. Welch and MacDaniels (13) observed that asphalt water emulsions and asphalt paints are durable and nonphytotoxic but fail to prevent bleeding and decay on field-grown apple trees. Marshall (4) indicated that asphalt dressings allow callus formation but do not prevent infection. Tamir (10) observed that asphalt dressings frequently cause blisters and stimulate decay. Shigo (7) found an unidentified fungus growing on asphalt-coated wounds of red maple and American elm and later observed that asphalt, orange shellac, and polyurethane varnish have no effect on invasion of wounds by microorganisms (8). Shigo and Wilson (9) observed that wounds treated with asphalt dressings readily become infected, often more severely than untreated wounds. Dye and Wheeler (3) treated filter paper with wound dressings, inoculated them with a spore suspension of *Stereum purpureum* placed in a stainless-steel cylinder on the filter paper, and noted that bitumen water emulsions are not toxic to the fungus.

Dye (2) used a filter paper disk technique to determine the toxic effects of acrylic emulsions alone and combined with captafol, mercuric oxide, captafol plus mercuric oxide, and Santar A on *S. purpureum.* May and Palmer (5) studied the effects of asphalt and asphalt-fungicide mixtures on *Ceratocystis fimbriata* f. *platani* in vitro. They grew the fungus, seeded at the points of an equilateral triangle on potato-dextrose agar plates for 4 days, then placed a small amount of paint on the agar in the center of the plate. The fungus was not affected by the asphalt wound paint.

May and Palmer (6) found that 11 fungal species were unaffected by asphalt varnish containing nine different fungicides. In one test they placed a drop of the test compound directly onto the center of the agar medium with the test fungus placed equidistant from the center at the points of an equilateral triangle. In other tests, material was applied to filter paper, which was allowed to dry for 24 hr and then divided into two lots. One lot was washed continuously in running water for 7 hr, and the other lot was placed, without washing, on the center of the medium, with the test fungus seeded to the plates in the same manner as in the first test. The reactions obtained by each of these techniques were similar. Dooley and Lagerstedt (1) found that asphalt paints alone and combined with methyl 2-chloro-9-hydroxyfluorene-9-carboxylate, methyl 9-hydroxyfluorene-9-carboxylate, and methyl 2,7-dichloro-9-hydroxyfluorene-9-carboxylate were not fungitoxic. In the same study a polyvinyl acetate paint was found to be fungitoxic but lost its fungitoxic effects when used with 2,4-D.

This paper reports in vitro studies with six commercially available asphalt tree wound paints. Two contained copper naphthenate, a proven fungicide. The objectives of this study were to determine if the paints initially inhibit wood decay fungi, to determine if nonfungitoxic paints provide a mechanical barrier to the fungi, and to suggest screening techniques that would be rapid and inexpensive for initial evaluation of tree wound paints.

MATERIALS AND METHODS

Fungitoxicity test. The method used was a modification of May and Palmer's technique (6). Fungitoxicity was evaluated against *Lenzites trabea* Pers. ex Fr. (Madison no. 617), *Poria placenta* (Fr.)

Table 1. Inhibition of mycelial growth of wood decay fungi by tree wound paints

Treatment	Concn. (%)	Days exposed	Number of disks overgrown by fungus/total disks: *Lenzites trabea*	*Stereum frustulatum*	*Poria placenta*	*Coriolus versicolor*
A. Asphalt solids	21.00	10				20/20
Petroleum distillates	23.00	12	20/20		20/20	
		15		20/20		
B. Asphalt solids	37.75	10				20/20
Petroleum distillates	19.25	12	20/20		20/20	
		20		20/20		
C. Asphalt solids	21.00	10				20/20
Petroleum distillates	22.00	12	20/20		20/20	
Pine oil	1.00	15		20/20		
D. Aliphatic hydrocarbons	61.00	12	20/20		20/20	
Petroleum distillates	37.00	15		18/18		8/20[a]
E. Copper naphthenate	10.00	14	0/20		0/20	
Asphaltum	40.00	15				0/20
Petroleum distillates	50.00	20		0/20		
F. Copper naphthenate	3.30	12	0/20			
Asphalt	37.40	14			0/20	
		15				0/18
		20		0/20		

[a]Test was inconclusive in results obtained.

Cooke (Madison no. 698), *Coriolus versicolor* (L. ex Fr.) Quél (Ithaca R-105-Sp), and *Stereum frustulatum* (Pers. ex Fr.) Echl. (FP 106073-S). Treatment ingredients, as they appear on the product labels, are listed in Tables 1 and 2.

Twenty milliliters of potato-dextrose agar (PDA) was poured into each 100 ×10 mm disposable petri dish and allowed to solidify. Each test fungus was transferred to the center of two dishes containing PDA and allowed to grow, at room temperature (20 ± 5 C), to the edge of the dish. Using a No. 4 cork borer, fungus-agar disks were cut and transferred, one per dish, to the center of the medium of five dishes per treatment per fungus.

Disks, 6 mm in diameter, of 202-grade filter paper were cut with an office punch and then sterilized in a glass petri dish. The sterile filter paper disks were individually dipped in the test paint and the excess was allowed to run off. Two treated disks of the same treatment were then transferred to the medium of each dish about 20 mm from and on opposite sides of the fungus-agar disk. The fungus was then allowed to grow at room temperature until the mycelium had grown past the treated disks or to the edge of the petri dish. Daily observations were made to determine growth rates of the fungi.

Table 2. Barrier action of six tree wound paints to two wood decay fungi

Treatment	Concn. (%)	Days exposed	Paint barriers penetrated/ total number treated	
			Lenzites trabea	*Stereum frustulatum*
A. Asphalt solids	21.00	9	12/12	
Petroleum distillates	23.00	23		12/12
B. Asphalt solids	37.75	19		11/12
Petroleum distillates	19.25	23	11/12	
C. Asphalt solids	21.00	9	12/12	11/12
Petroleum distillates	22.00			
Pine oil	1.00			
D. Aliphatic hydrocarbons	61.00	19		12/12
Petroleum distillates	37.00	23	12/12	
E. Copper naphthenate	10.00	23	0/12	0/12
Asphaltum	40.00			
Petroleum distillates	50.00			
F. Copper naphthenate	3.30	23	0/12	0/12
Asphalt	37.40			

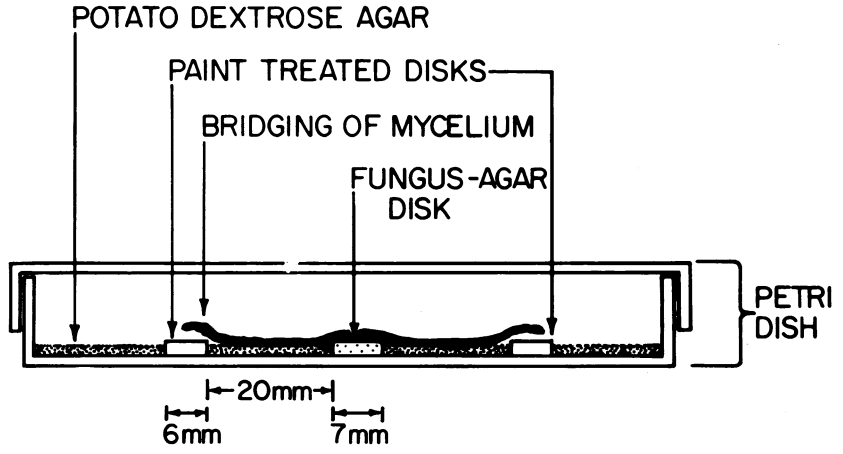

Fig. 1. Petri dish containing 20 ml of potato-dextrose agar, a fungus-agar disk in the center, and paint-treated filter paper disks on opposite sides of the fungus-agar disk. Note bridging of mycelium over the treated disks.

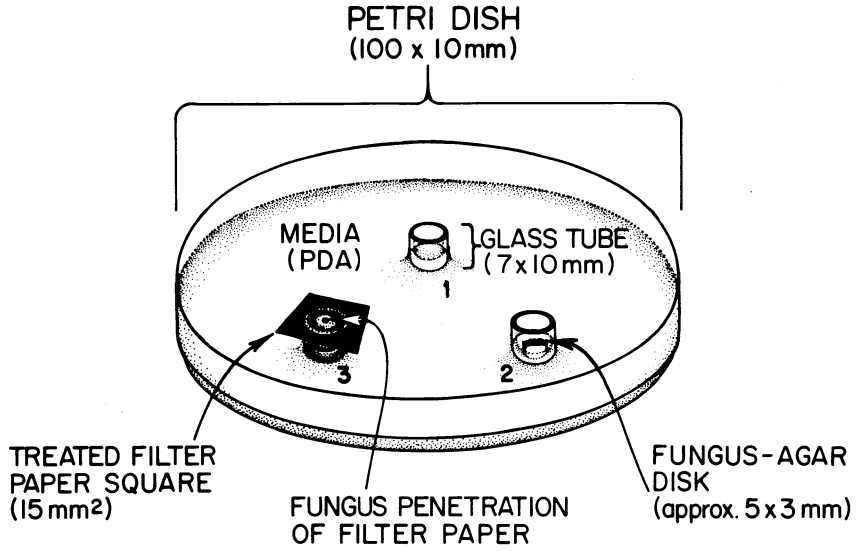

Fig. 2. Steps in the barrier test.

Fungitoxicity data collection. The zone of inhibition in millimeters from the treated disk to the leading edge of the fungal colony was recorded. In some instances the fungus grew to the edge of the treated filter paper disk and stopped, suggesting that the chemical was fungistatic if not fungitoxic. The number of treated filter paper disks overgrown by the fungus per total number of disks was recorded.

Care should be taken to distinguish between overgrowth and bridging followed by collapse of the mycelium. Bridging occurs when the fungus grows over the paint without actually touching it (Fig. 1). Daily observation allows early detection of bridging. If bridging is unnoticed, the results may be misinterpreted because bridges often collapse onto the treated material.

Barrier test. Prospective wound paints should be tested for barrier action. If the paint is fungistatic or fungitoxic and the fungus is unable to penetrate the paint film supported by filter paper, there is little doubt that the paint provides a inhibitive barrier. If the wound paint is not inhibitory, however, the question that remains is whether the paint will provide a mechanical barrier to the fungus. The barrier test was designed to answer this question and is illustrated in Fig. 2.

Tree wound paints were evaluated for barrier action against *L. trabea* and *S. frustulatum* by the same treatments used in the fungitoxicity tests. Twenty milliliters of PDA was poured into each of four dishes for each fungus per treatment. While the medium was still liquid, three sterilized glass tubes, 7 mm long and 10 mm in diameter, were placed with one open end up into each dish equidistant from the center and each other. The medium was allowed to solidify for at least 12 hr to allow for contraction.

A No. 2 cork borer was used to cut

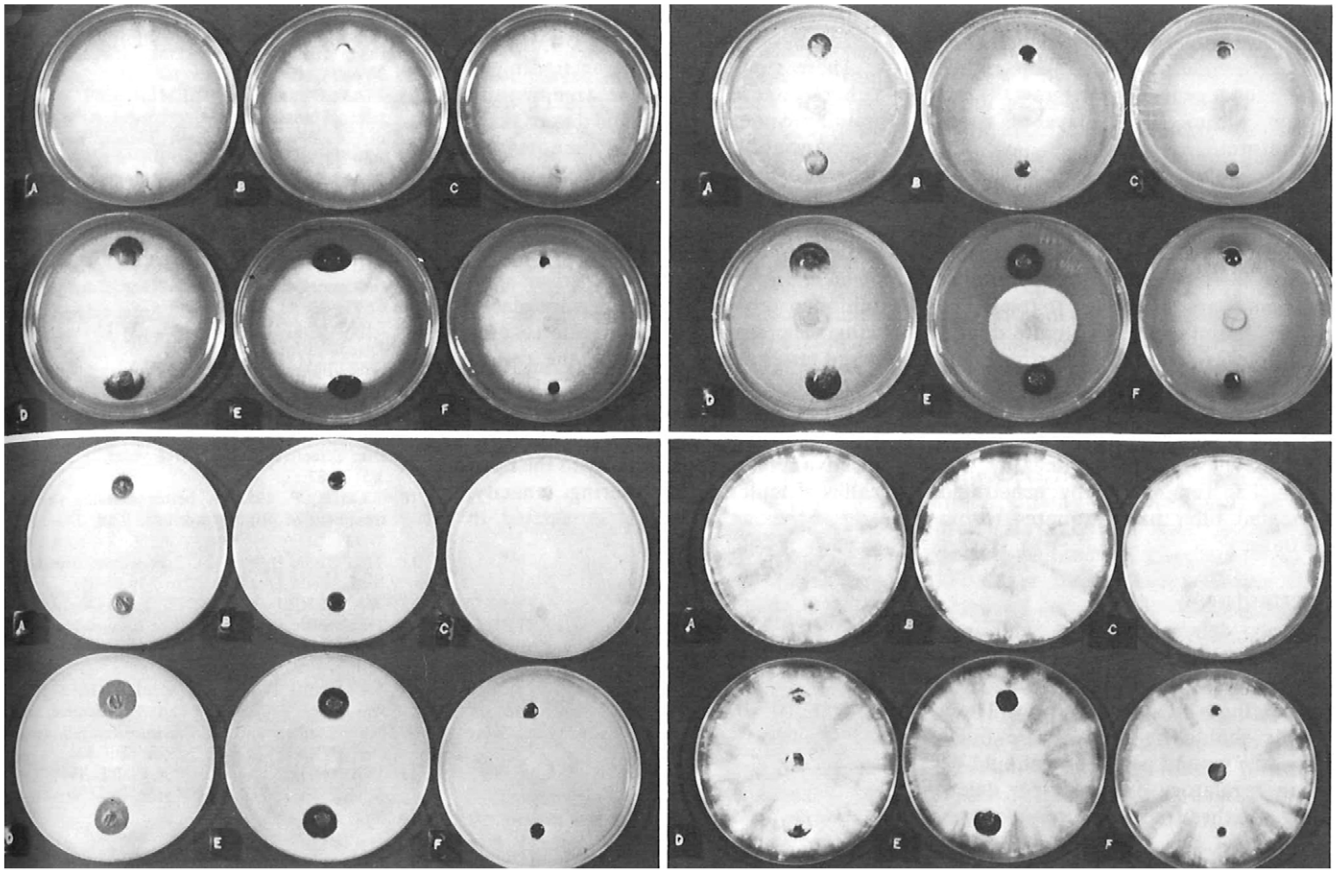

Fig. 3. Fungus growth on potato-dextrose agar exposed to filter paper disks treated with: **(A)** 21% asphalt solids and 23% petroleum distillates; **(B)** 37.75% asphalt solids and 19.25% petroleum distillates; **(C)** 21% asphalt solids, 22% petroleum distillates, and 1% pine oil; **(D)** 61% aliphatic hydrocarbons and 37% petroleum distillates; **(E)** 10% copper naphthenate, 40% asphaltum, and 50% petroleum distillates; **(F)** 37.4% asphalt and 3.3% copper naphthenate. **Top:** (left) *Lenzites trabea* exposed for 12 days (bridging is shown in E) and (right) *Stereum frustulatum* exposed for 15 days. **Bottom:** (left) *Poria placenta* exposed for 15 days and (right) *Coriolus versicolor* exposed for 15 days.

fungus-agar disks from fresh fungus cultures grown in petri dishes. One fungus-agar disk was transferred into each glass tube; the tubes within each petri dish received the same fungus. The fungus-agar disks were placed in contact with the agar in the tubes. Sterilized filter paper squares (15 mm^2) were dipped individually into the wound paints. The excess paint was allowed to run off and the treated papers were placed on top of the glass tubes, approximately 3 mm above the fungus-agar disk. One paint was tested in each dish. The fungus was allowed to grow at room temperatures for a maximum of 25 days after treatment. Tests were terminated earlier if the fungus penetrated the treated filter paper squares.

Results were recorded as the number of treated filter paper squares penetrated by the fungus per total number of treated filter papers. Twelve replicates were used.

RESULTS

Of the six asphalt tree wound paints, only those treatments that contained 10 and 3.3% copper naphthenate inhibited fungal growth (Table 1). Treatment E, (Fig. 3, top, left) illustrates bridging, as does Fig. 1. The paints containing copper naphthenate showed no distinct zone of

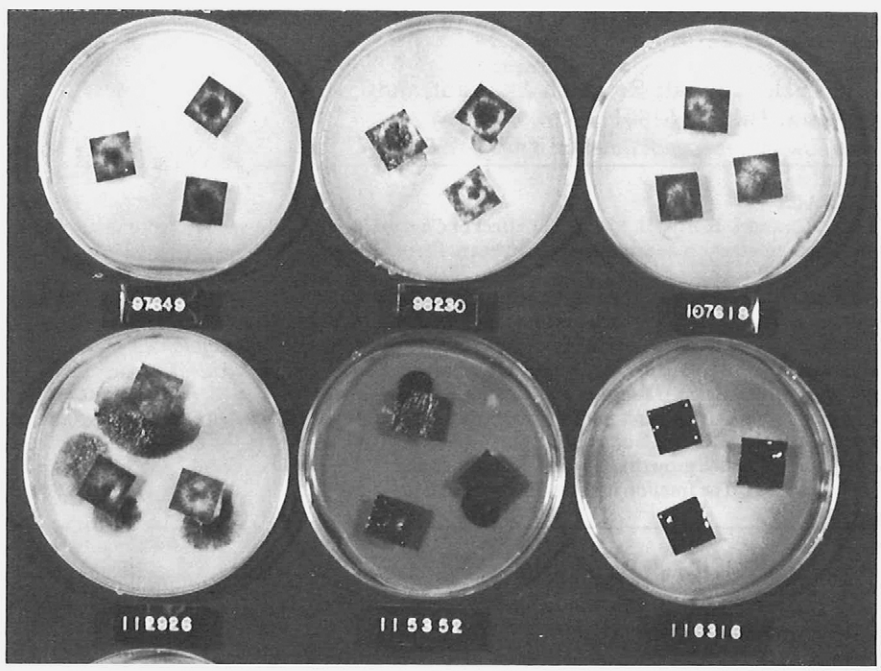

Fig. 4. Barrier test showing penetration of *Stereum frustulatum* through treated filter paper squares after 23-day exposure. Treatments are: **Top:** (left to right) 21% asphalt solids and 23% petroleum distillates; 37.75% asphalt solids and 19.25% petroleum distillates; 21% asphalt solids, 22% petroleum distillates, and 1% pine oil. **Bottom:** (left to right) 61% aliphatic hydrocarbons and 37% petroleum distillates; 10% copper naphthenate, 40% asphaltum, and 50% petroleum distillates; 37.4% asphalt and 3.3% copper naphthenate.

inhibition with *L. trabea, P. placenta,* and *C. versicolor,* and only a small zone with *S. frustulatum* (Fig. 3, top, right). The test fungi generally overgrew the test medium within 10–20 days at room temperature.

All six wound paints were evaluated for barrier action to *S. frustulatum* and *L. trabea.* Only paints containing copper naphthenate provided a barrier (Table 2), but, of course, large amounts of copper naphthenate are phytotoxic to trees. The remaining paints were not fungitoxic and did not act as mechanical barriers. The reaction to the paints of *S. frustulatum* (Fig. 4) and *L. trabea* were the same, ie, all paints that did not contain copper naphthenate were penetrated by the fungus. The fungus usually penetrated the treated filter paper squares within 9–23 days.

DISCUSSION

In vitro determination of inhibitory and mechanical barrier action of tree wound paints to fungi can be determined by the methods used in this study. These methods should be used before field-testing tree wound paints and should be used in regulatory programs for determining whether tree wound paints have initial inhibitory or mechanical barrier action.

These studies confirm the finding of others that asphalt-type tree wound paints are not fungitoxic and do not act as mechancial barriers to Hymenomycete mycelia. Addition of 3.3 and 10% copper naphthenate to these paints provides an inhibitory barrier.

In this study, some fungi, eg, *Penicillium* spp. and *Aspergillus* spp., inhibited growth of certain wood decay fungi. Therefore, if contamination occurs when using these methods, the results should be considered invalid.

All in vitro methods used to evaluate tree wound paints have inherent disadvantages. Phytotoxicity to the host, callus stimulation, weathering, tenacity, and longevity cannot be evaluated in vitro.

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