Decay Resistance of Red Pine Wood Chips Enriched with Oleoresin

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ABSTRACT

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Red pine (*Pinus resinosa*) trees growing in Wisconsin were treated with paraquat to induce synthesis and deposition of high oleoresin contents (lightwood production), or with water (controls). The trees were harvested 2 yr later and wood chip samples containing average levels of nonvolatile oleoresins of 3.9 (controls), 9.7, and 15.9% were obtained. These samples were subjected to decay by the typical wood decay fungi, *Phanerochaete chrysosporium*, *Poria subvermispora*, *Gloeophyllum trabeum*, an unknown basidiomycete (ME-493), and by the soft-rot fungus, *Papulospora* sp. Weight losses caused by each of the test fungi were consistently less in the lightwood than in the normal wood chips. Generally, little difference in decay resistance was found between chips containing average nonvolatile oleoresin contents of 9.7 and 15.9%. Because of the greater decay resistance of lightwood, its initial dryness, and fewer living parenchyma cells, wood chip storage piles consisting predominantly of lightwood should undergo reduced biological deterioration and initial heating.

Field trials have demonstrated the marked stimulation of oleoresin (turpentine and rosin) synthesis and deposition in wood, commonly called lightwood, of *Pinus* species by the systemic application of bipyridilium salts (ie, paraquat and diquat) (10) or of ethylene-releasing compounds, eg, ethrel (18). It is also apparent that treatment with bipyridilium salts is economically attractive (4,8) and is likely to be adopted by both the pulp and paper and naval stores industries in future forest management plans. A small portion of the increased oleoresin content will be preextracted from the trees for the naval stores industry. However, the bulk of the oleoresin will most likely be recovered as tall oil at the end of the pulping process. Therefore, coniferous pulp chips in the market will in the near future reflect this change in oleoresin content, especially those from the southeastern United States. Chip piles will contain increasing amounts of chips with oleoresin beyond the 3–4% normally encountered in pine sapwood. In addition to the increased rosin in lightwood, there is a concomitant increase in turpentine content.

These differences in chip constituents will certainly change some aspects of handling chips in storage. Of special interest is the question of how the increased content of oleoresin, both volatile and nonvolatile, will affect the ability of the chips to resist microbial degradation. Weight losses as high as 1.4% per month have been reported for typical normal southern pine species (7). If the increased oleoresin content in the chip piles can reduce microorganism degradation, this reduction will result in reduced losses of fatty acids as well as of cellulose fiber, and in maintenance of pulp brightness. Amburgey et al (1) found that lightwood-modified southern pine roundwood had somewhat greater resistance than did normal roundwood to decay by *Gloeophyllum trabeum* (Pers.) Murr. Unfortunately, the concentration of oleoresins in the test woods was not determined. In addition, Beal et al (2) found in field trials that similar lightwood was more resistant to deterioration by subterranean termites and marine borers, as well as by decay fungi. Resin accumulations in roots of living white spruce trees were found to inhibit infection by some heart-rot fungi (17).

The objective of the present study was to determine the effect of increasing oleoresin contents in pine wood on severity of decay by important wood-chip-storage fungi. This information would, in turn, permit determination of the storage conditions required for chips obtained from lightwood.

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MATERIALS AND METHODS

Lightwood induction and analysis. Plantation-grown, 20-yr-old red pine (*Pinus resinosa* Ait.) trees in Wisconsin were treated in early spring of 1976 with aqueous solutions of either 2 or 4% commercial (Chevron) paraquat, or with water alone (controls). Solutions were applied to the tree boles in horizontal ax gashes, located 0.75 m above ground level, at the rate of 2 ml/cm of gash for half the circumference of the tree.

Wood samples of varying oleoresin content were obtained from the treated trees in spring of 1978. Because a gradient of oleoresin content is formed in treated trees (18), with oleoresin concentrations decreasing with increasing distance from the point of treatment, 25-cm bolts were removed from paraquat-treated trees at distances of both 10 and 150 cm from the point of treatment. A similar-sized bolt was also removed from each of the water-treated (control) trees at a height of 0.75 m above ground level. All bolts were stored at −20 C until removed for analyses. Each bolt was subsequently debarked and converted to chips about 16 mm diameter and the chips were thoroughly mixed. Representative groups of chips were then removed from each sample for moisture determinations and for replicate analysis of their total nonvolatile oleoresins. The latter process was accomplished by Soxhlet extractions with ether for 20 hr. For details see Wolter and Zinkel (19) and Zinkel (20).

Decay testing (chip preparation). Chip samples from different heights on treated trees and from the control trees were individually screened to remove fines and small wood splinters. The remaining chips were air-dried at room temperature for 3 days and conditioned to constant weight at about 27 C and 70% relative humidity. Samples of the conditioned chips (20.00–20.05 g) were placed in glass cylinders similar to those used by Eslyn (5) (37 mm inside diameter, about 30 cm long, plugged at both ends with a rubber stopper pierced by glass tubing) (Fig. 1). Rubber tubing was attached to each glass tube to permit sealing of the glass cylinder. Because autoclaving or steaming of the chips in preliminary tests had a marked effect on their oleoresin content, gas sterilization was deemed advisable. (All volatile constituents, primarily turpentine, are affected by any sterilization procedures; therefore, that component of the oleoresins could not be evaluated in these tests.)

It has been shown (12) that wood with a moisture content between 10 and 50% is most effectively sterilized by ethylene oxide; hence, prior to sterilization, chip moisture content was raised by

inserting water-saturated sponges into both ends of each cylinder and leaving them in place for 48 hr. The sponges were removed, and the lower one was replaced with cotton wadding. Ten milliliters of ethylene oxide liquid was poured onto the cotton in each cylinder. Volatilization of the ethylene oxide produced a positive pressure which required venting of the cylinders until the pressure was reduced. The cylinders were then sealed for 48 hr because preliminary experiments had shown this time to be sufficient for complete sterilization of the chips. Following sterilization, filtered air was flushed through the cylinders for 72 hr to eliminate residual ethylene oxide.

To determine if control or lightwood samples absorbed moisture differentially, cylinders of chips were weighed prior to and following a 3-day exposure to water-saturated air.

Decay testing (preparation of inoculum). The five test fungi used were: *Phanerochaete chrysosporium* Burds. (ME-446), a thermotolerant, white-rot fungus; *Poria subvermispora* Pilat (ME-485), a white-rot fungus; a basidiomycete (ME-493), culturally nondescribed, but (based on its positive reactions on both gallic and tannic acids) probably a white-rot fungus; *Gloeophyllum trabeum* (Pers.) Murr. (MAD-617) a brown-rot fungus; and *Papulospora* sp. (ME-PC-19) a soft-rot fungus.

Except for *G. trabeum*, all test isolates had been isolated from wood chip storage piles, and all had been shown in previous laboratory tests to be capable of causing appreciable weight losses in wood.

Inocula of *P. chrysosporium*, *G. trabeum*, and *Papulospora* sp. were prepared as follows: flasks containing 50 ml of Abrams' solution (an aqueous solution containing (per liter): 3 g NH_4NO_3, 2 g K_2HPO_4, 2.5 g KH_2PO_4, and 2 g $MgSO_4 \cdot 7H_2O$) plus 0.5 g rolled oats were autoclaved for 20 min at 1.05 kg/cm^2 (15 lb), then cooled and inoculated with two pieces (3 mm^2) of one of the above fungi. The flasks were then placed on a shaker and maintained at 27 C and 70% relative humidity for 10–16 days, depending upon the rate of growth. *P. subvermispora* and the unknown basidiomycete (ME-493) produced little or no growth on Abrams' solution; thus, they were grown instead in a medium consisting of 50 ml of a 0.5% aqueous solution of glucose containing 0.5 g of rolled oats. Once inoculated, these flasks were treated like those containing the other three fungi.

Decay testing (inoculation and incubation of wood). Inoculation was accomplished by removing the rubber stopper from one end of the cylinder and aseptically pouring a flask of inoculum, consisting of fungal pellets plus nutrient solution, over the wood chips in each cylinder. The cylinders were then shaken to distribute the inoculum and the nutrient solution. Excess solution was drained from the bottom of the cylinder. To permit better gas exchange, the rubber stopper at the top of each cylinder was replaced with a sterilized foam plug (Fig. 1). All cylinders were stored at 27 C except those containing the thermotolerant *P. chrysosporium*. These were incubated at 45 C. At the end of 6 wk of incubation, 50 ml of sterile distilled water were added to each cylinder to prevent drying out of the chips. After 12 wk of incubation the chips were removed, reconditioned to constant weight at 27 C and 70% relative humidity, reweighed, and the weight losses calculated. All tests were replicated five times.

RESULTS

Rosin and moisture contents of wood chips. Rosin and moisture contents of the different chip samples are provided in Table 1. Trees not treated with paraquat yielded chips containing an average of 3.9% nonvolatiles (rosin). Trees treated with 2% paraquat contained average rosin contents of 15.9 and 9.7% at 10 and 150 cm, respectively, from the point of treatment. Treatment with 4% paraquat appeared to reduce rosin production 10 cm from the treatment point, but had little or no effect upon production rate 150 cm from the treatment site compared with those values in samples from trees treated with 2% paraquat (Table 1).

Effect of rosin content upon wood decay. Extent of decay, as measured by weight losses due to fungal degradation, was greatest in wood chips obtained from control trees, which contained an average of 3.9% rosin (Fig. 2). All five test fungi reduced the weight of the control wood at an appreciably greater rate than they did the lightwood, which contained a higher rosin content. However, the extent to which rosin protected against decay varied with the causal fungus. Decay caused by the unidentified basidiomycete (ME-493) decreased from an average of 25.3% with control wood to 16.7–18.7% in lightwood; decay caused by *G. trabeum* (617) dropped more drastically from 18.9% down to 2–3% (Fig. 2).

No substantial decrease in decay losses resulted from increasing

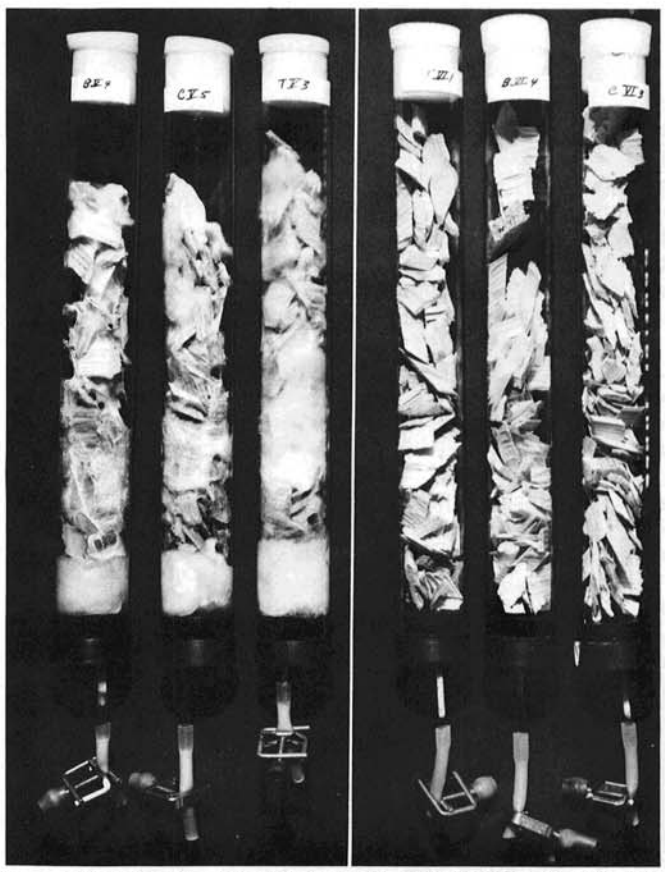

Fig. 1. Decay of red pine chips by a chip pile basidiomycete (isolate ME-493). Decay chambers (cylinders) following inoculation and incubation. Chips in the three cylinders on the left were inoculated, while those in the three on the right were treated with sterile media (controls).

TABLE 1. Rosin and moisture contents of red pine wood chip samples[a] at varying distances from point of treatment

Tree treatment (%) paraquat	Rosin content (%)			Moisture content (%)[b]		
	0	10	150[c]	0	10	150[c]
0 (control)	3.9(0.02)[d]	...	...	127.7(0.62)	...	...
2	...	15.9(0.54)	9.7(0.90)	...	84.8(0.10)	101.0(0.64)
4	...	7.9(0.66)	9.1(0.34)	...	61.9(0.88)	61.4(1.70)

[a] Each sample was obtained from two trees, pooled, and subjected to six replicate analyses.
[b] Based on oven-dry weight and corrected for rosin content.
[c] Distance (cm) from point of treatment.
[d] Standard deviation in parentheses.

rosin content from 9.7 to 15.9% (Fig. 2). In one case involving *P. subvermispora* (485), weight losses in chips containing 15.9% oleoresins were actually higher than in those containing 9.7%.

The ability of wood chips to absorb moisture during reconditioning was not affected by the oleoresin content. Therefore, the differences in weight losses shown in Fig. 2 were associated with differences in rosin content rather than in moisture content.

DISCUSSION

Red pine wood chips containing an average of 9.7% or more rosin were more resistant than normal wood to deterioration by chip storage decay fungi. Lower fiber losses from fungal degradation should thus be expected in wood chip storage piles consisting largely of lightwood. The question arises, however, whether the apparent increase in decay resistance of lightwood may be due to the inability of the test fungi to metabolize rosins. In this regard, Hart et al (6) found that wood blocks artificially impregnated with resin acids and then subjected to decay by *C. versicolor* lost considerably less weight than did nonimpregnated blocks. The difference could not be attributed to the inability of the fungus to utilize resin acids. Prior (9), however, attributed decreased fungal growth in living pines affected by resinosus to mechanical blocking of tracheids rather than to fungitoxicity of oleoresin. He found by laboratory tests that, although purified resin acids were toxic to the root pathogen *Heterobasidion annosum* (Fr.) Bref., oleoresin itself was nontoxic. Also, pulverizing resinous wood made it as susceptible to decay as normal wood.

Other compounds that affect fungal development are associated with resinosis in injured or decaying pine trees. There are conflicting views as to the relative importance of these compounds in protecting wood from fungal invasion. Prior (9), for example, considered pinosylvins, synthesized in response to cell injury, to be primarily responsible for containment of fungal invasion in pines. Shain (11), however, found that although pinosylvins may contribute to decay resistance in resinous wood ("reaction wood") other compounds (which he called phytoalexins) were responsible for impeding fungal advance in pine trees. Investigation of the chemical components of lightwood was not a part of the present study, hence the role of individual compounds in decay resistance of this wood was not determined. In addition, the study was not designed to determine the manner, chemical or mechanical, in which oleoresin contributed to reduced decay susceptibility of lightwood.

In addition to its greater resistance to decay associated with increased content of nonvolatile oleoresins, lightwood could also be expected to contribute to increased durability of chip storage piles owing to the following attributes:

Death of living cells occurs in those areas of a tree affected by lightwood-inducing treatments. Because an initial rise in temperature of a chip pile is partially attributable to the respiration of living parenchyma cells (14), the heat rise in stored lightwood chips should be slower. This slower rise would, in turn, inhibit development of thermophilic microorganisms and consequent deterioration and further heat increases due to their activities. Increased activity of mesophilic fungi might prevail under the cooler conditions and thus offset the decrease in thermophilic activity.

Volatile components of oleoresin have been found, in part, to inhibit growth of decay fungi (3,16) and blue-stain fungi (3), when these were tested in sealed containers (9). Although not measured directly in the present study, most of the volatiles were assumed to have been lost from the wood chip samples during sterilization. Hence, volatiles probably contributed little or nothing to the decay resistance of the lightered wood chips. Turpentine losses in southern pine wood chips during outside storage have been found to vary considerably between tests. Somsen (13) found that turpentine content remained constant for 7 wk before decreasing rapidly until 80% was lost. However, Springer et al (15) found that 45% of the turpentine was lost during the first 7 days of storage. Volatiles in stored lightwood chips, although reduced, might inhibit decay fungi during the early stage of storage and, hence, also reduce loss of fiber in the stored wood.

Wood obtained from trees treated to produce lightwood has a much lower moisture content than that obtained from untreated healthy trees (Table 1). However, the level of moisture remaining in the former was still sufficient for decay to occur. Thus, the lightwood would have to approach the fiber saturation point before significant inhibition of fungal development would occur.

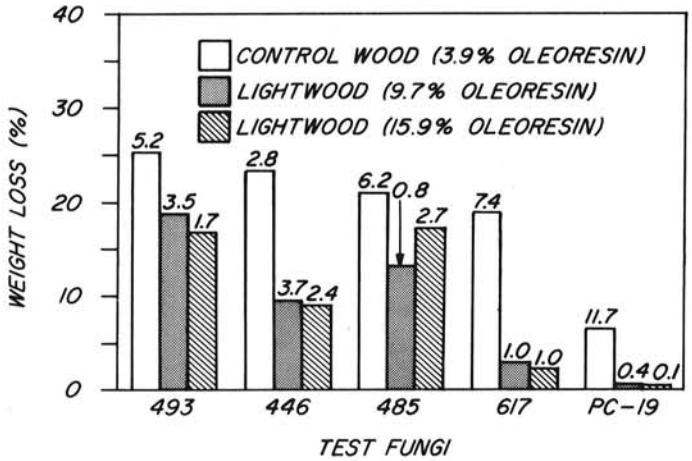

Fig. 2. Effect of nonvolatile oleoresin (rosin) contents in red pine chips upon decay potential of the five wood-rotting fungi, an unknown basidiomycete (493); *Phanerochaete chrysosporium* (446); *Poria subvermispora* (485); *Gloeophyllum trabeum* (617); and *Papulospora* sp. (PC-19). Weight losses are based on averages of five replications. Standard deviations are indicated on the top of each bar.

LITERATURE CITED

1. Amburgey, T. L., Carter, F. L., and Roberts, D. R. 1978. Resistance of wood from paraquat-treated southern pine trees to termites and the fungus *Gloeophyllum trabeum*. Wood Sci. 10:187-192.
2. Beal, R. H., Bultman, J. D., Amburgey, T. L., and Roberts, D. R. 1979. Resistance of wood from paraquat-treated southern pines to subterranean termites, decay fungi, and marine borers. For. Prod. J. 29:35-38.
3. Cobb, F. W., Jr., Krstic, M., Zavarin, E., and Barber, H. W., Jr. 1968. Inhibitory effects of volatile oleoresin components on *Fornes annosus* and four *Ceratocystis* species. Phytopathology 58:1327-1335.
4. Collier, T. 1977. Feasibility of petrochemical substitution by oleoresins. Pages 172–187 in: Proc. Lightwood Res. Coord. Counc., Jacksonville, FL.
5. Eslyn, W. E. 1973. Evaluating chemicals for controlling biodeterioration of stored wood chips. For. Prod. J. 23:21-25.
6. Hart, J. H., Wardell, J. F., and Hemingway, R. W. 1975. Formation of oleoresin and lignins in sapwood of white spruce in response to wounding. Phytopathology 65:412-417.
7. Lindgren, R. M., and Eslyn, W. E. 1961. Biological deterioration of pulpwood and pulp chips during storage. Tappi (Tech. Assoc. Pulp Pap. Ind.) 44:419-429.
8. McSweeney, E. E. 1977. The economics of paraquat induced lightwood formation. Pages 167–171 in: Proc. Lightwood Res. Coord. Counc., Jacksonville, FL.
9. Prior, C. 1976. Resistance by Corsican pine to attack by *Heterobasidium annosum*. Ann. Bot. 40:261-279.
10. Roberts, D. R. 1973. Inducing lightwood in pine trees by paraquat treatment. U.S. For. Serv. Res. Note SE-191.
11. Shain, L. 1967. Resistance of sapwood in stems of loblolly pine to infection by *Fomes annosus*. Phytopathology 57:1034-1045.
12. Smith, R. S. 1968. Effect of moisture content on the sterilization of wood, under vacuum, by propylene and ethylene oxides. Can. J. Bot. 46:299-303.
13. Somsen, R. A. 1962. Outside storage of southern pine chips. Tappi (Tech. Assoc. Pulp Pap. Ind.) 45:623-628.
14. Springer, E. L., and Hajny, G. J. 1970. Spontaneous heating in piled wood chips. Tappi (Tech. Assoc. Pulp Pap. Ind.) 53:85-86.
15. Springer, E. L., Feist, W. C., Zoch, L. L., Jr., Hajny, G. J., and McAliley, J. E. 1974. Evaluation of two chemical treatments in outside

chip piles. U.S. For. Serv. Res. Pap. FPL-243.
16. Värsälä, L. 1974. Effects of terpene compounds on the growth of wood-decomposing fungi. Ann. Bot. Fenn. 11:275-278.
17. Whitney, R. D., and Denyer, W. B. G. 1969. Resin as a barrier to infection of white spruce by heart-rotting fungi. For. Sci. 15:266-267.
18. Wolter, K. E. 1977. Ethylene—potential alternative to bipyridilium herbicides for inducing lightwood in red pine. Pages 90–99 in: Proc. Lightwood Res. Coord. Counc., Jacksonville, FL.
19. Wolter, K. E., and Zinkel, D. F. 1976. Metabolic, hormonal, photosynthetic and lightering response of red pine treated with paraquat. Pages 31–39 in: Proc. Lightwood Res. Coord. Counc., 20–21 January 1976, Jacksonville, FL.
20. Zinkel, D. F. 1975. Tall oil precursors—an integrated analytical scheme for pine extractives. Tappi (Tech. Assoc. Pulp Pap. Ind.) 58:109-111.