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Decay Resistance in Conifer Seed Cones: Role of Resin Acids as Inhibitors of Decomposition by White-Rot Fungi

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Summary

Resin acids in the diethyl ether extracts of *Picea glauca, Pinus ponderosa* and *Pinus banksiana* seed cones were identified by gas-liquid chromatography of their methyl-ester derivatives. For these seed cones, abietic, dehydroabietic and isopimaric acids comprised 63.7-80.5 % of the total resin acids identified. In P. *banksiana,* the resin acid composition of the seed cones was shown to be significantly different from that in either the leaves, bark or wood. Investigation of the role of resin acids in the apparent decay resistance of woody conifer tissues to white-rot fungi involved the incorporation of abietic, dehydroabietic and isopimaric acids individually, or as a 1 : 1 : 1 mixture, into decay susceptible sweetgum *(Liquidambar styraciflua)* wood test blocks which were subsequently exposed to cultures of *Irpex lacteus* and *Trametes versicolor.* Inhibition of decay by /. *lacteus* was observed for test blocks treated with abietic and dehydroabietic acids, but not isopimaric acid. With *T. versicolor,* only those test blocks treated with abietic acid showed less decay when compared with controls. Comparisons of the decay levels with the moisture contents of resin acid treated test blocks, and the analyses of residual resin acid contents of decayed blocks, indicate that resin acids provide decay resistance by their water repellency and inherent decay resistance rather than general toxicity.

Introduction

Regeneration by conifers involves the production of seeds in structures commonly identified as cones. Although the seeds of conifers are not enclosed in an ovary but situated on the top surface of ovuliferous scales, they nevertheless are protected from the external environment owing to the intricate structure of the seed cone. For many pines, once the seeds have matured, they are released from the cones which in turn are shed and incorporated into the woody litter layer. On the forest floor, seed cones appear to be more persistent than other woody litter *(e.g.* twigs, branches) which has been shown to decay 2-3 times more rapidly (Edmonds 1987). This is not surprising since seed cones need to be resilient to protect the developing seed crop.

When faced with an attack by a fungal pathogen, woody plants may show resistance through active and/or passive defense mechanisms involving the formation of physical and/or chemical barriers (Merrill 1992; Shigo 1984). In this regard, substances actively or passively deposited within the externally exposed tissues of a pine tree *(e.g.* bark, leaves, cones) can be inhibitory or even toxic to the advancing fungus. In pines, bark tissues contain presumably decay-resistant polymers *(e.g.* suberin, lignin, tannins) and a variable spectrum of potentially toxic low molecular weight extractives (Kolattukudy and Espelie 1985; Woodward 1992). Foliar diseases, most often caused by fungi, are apparently resisted by the hydrophobic barrier provided by the cuticle (Adaskaveg 1992; Kolattukudy and Espelie 1985). Not only do pine trees draw a chemical line of defense along their peripheral tissues, but also from within through the deposition of fungitoxic extractives *(e.g.* pinosylvins) during heartwood formation (Hillis 1987; Kuc and Shain 1977; Rishbeth 1972).

Ongoing concerns regarding the toxicity and environmental hazards associated with commercial wood preservatives has provided the stimulus to gain a greater understanding of the mechanisms of decay resistance employed in nature. Extractives from bark (Harun and Labosky 1985; Laks ***et al.*** 1988) or naturally durable wood (Smith ***et*** *al.* 1989a, 1989b) have been utilized to improve the durability of decay susceptible woods. Following up on the apparent natural durability of seed cones, recent research efforts have indicated that these structures may provide an alternative source of fungistatic extractives (Micales ***et al.*** 1994). Unfortunately, detailed information about the nature of the extractives present in

seed cones is lacking. Indeed, our understanding of the chemistry of seed cones is so poor that we can only presume that the insoluble cell wall constituents *(e.g.* hemicelluloses, lignin) are similar to those in wood. Further studies are needed to chemically characterize conifer seed cones in order to realize their potential value as a renewable source of extractives (and biomass) that can be collected without harvesting trees.

Recently, resin acids, an important group of extractives found in conifers, were identified in seed cones (Haflzoglu and Holmbom 1987; Micales *et al.* 1994). Common to pines, these diterpenoids are primary constituents of the oleoresin produced in the resin canals of the needles, bark and wood (Croteau and Johnson 1985). As an active means of defense, oleoresin production is induced upon wounding (Cheniclet 1987; Gref and Ericsson 1985; Hart *et al.* 1975) especially when the wounds are infected with a fungal pathogen (Cheniclet 1987). Oleoresin and resin acids incorporated into agar media have been shown to inhibit fungal growth (Prior 1976; Shain 1971). Specific resin acid types *(i.e.* abietane, pimarane) have exhibited significantly different levels of fungal inhibition (Micales *et al.* 1994). Unfortunately, the extent to which resin acids inhibit fungal growth on a natural substrate *(e.g.* wood) remains unclear.

To explore the possible role that resin acids may fulfill in decay resistance exhibited by conifer seed cones, we first investigated the resin acid composition from various sources of seed cones. Comparisons were then made with the leaves, bark and wood. Specific resin acids of the abietane and pimarane types were then selected in order to determine their ability to inhibit the decay of a woody substrate.

Materials and Methods

Plant materials

Seed cones of ponderosa pine *(Pinus ponderos(l* Laws.), white spruce *(Picea glauca* (Moench) Voss) and jack pine *(Pinus banksiana* Lamb.), all of which matured during fall of 1992, were obtained from sources in Wisconsin. After rinsing with distilled water, *P. ponderosa* and *P. g/auca* cones were dried in a warm oven (35 °C). The serotinous cones of *P. banksiana* were soaked in distilled water for 30 minutes and \$en warmed in an oven (50 °C) to initiate scale opening and concomitant seed release. After the removal of any attached seeds, the dry cone tissues were ground in a Wiley mill (Model 2, 4mm mesh).

Samples of *P. banksiana* leaves, bark and wood were obtained from a tree *(ca.* 15 years old) harvested from the same stand in which the respective cones were collected. Individual tissues were rinsed with distilled water, frozen in liquid N., freeze-dried and then ground as above.

Isolation of extractives

Dry tissues were extracted with diethyl ether for 24 hours using a Soxhlet apparatus. Subsequent extractions with solvents of increasing polarity (acetone, methanol, water) were carried out in the same manner. All extracts obtained were dried *in vacuo,* purged with N2 and stored at -20°C.

Analyses of resin acids

Separation of the weak acid components *(e.g.* resin and fatty acids) from the ether extracts was accomplished by ion exchange chromatography over DEAE-Sephadex A-25 (Pharmacia) according to the procedure of Zinkel (1983). This involved the application of an aliquot (10-l4mg) of an ether extract, dissolved in a small amount of diethyl ether: methanol: water (90: 10: 1), to a column (5 mm x 5 cm) of the packing material equilibrated in the same solvent mixture. Following elution to remove neutral species, weak acid components were eluted with the above solvent system saturated with CO2. After drying *in vacuo,* the weak acid fraction was dissolved in diethyl ether: methanol (9 : 1) and methylated with etherial diazomethane (Zinkel and Han 1986).

Identification of the resin acids present was carried out by gas-liquid chromatography (GLC) using established chromatographic conditions (Han and Zinkel 1990, 1991). Methylated samples were dissolved in *tert*-butyl methyl ether and separated over DB-1 (J & W Scientific) and BDS (Supelco) columns using a Hewlett Packard model 5880A series gas chromatograph equipped with a 5880A series GC terminal (Level Four).

Determination of decay inhibition

Decay resistance imparted by resin acids was determined by a modified version of the ASTM (1992) procedure used to test the efficacy of wood preservatives. Fungal cultures of *Trametes versicolor* (L. : Fr.) Pilat (MAD697) and *lrpex lacteus* (Fr. : Fr.) Fr. (MAD7328) were grown in bottles (French square, 225 mL) containing moist soil and sapwood (sweetgum, *Liquidambar styraciflua* L.) feeder strips. Prior to the introduction of the test specimens, the soil bottle cultures were maintained in darkness at constant temperature (27 °C) and relative humidity (70%) for 4 weeks.

Sapwood *(sweetgum)* test specimens (3 mm x 6 mm x 6 mm) were oven dried (103 ± 2 °C), weighed, and then treated with either 50, 100 or 150mM solutions of pure *(ca.* 99%) isopimaric, abietic or dehydroabietic acids in ethanol. Treatment solutions of isopimaric acid: abietic acid: dehydroabietic acid (1: 1: 1) having total resin acid concentrations of 50, 100 and 150mM were also used. During treatment, the test blocks were distributed along the bottom of a beaker in a vacuum desiccator and exposed to a partial vacuum of 40 mm Hg for 30 minutes after which the treatment solution was introduced thereby submerging and infiltrating the specimens. After full release of the vacuum, the wet blocks were dabbed with a tissue, Weighed and then air dried. Estimates of the treatment retentions were made using the wet weights. For the controls, wood blocks were treated with a solution of pentachlorophenol (50mM) in ethanol or ethanol alone.

After steam sterilization (107 °C, 30min.), 3 test blocks were aseptically transferred into each culture bottle. Following 5 weeks of incubation, the test blocks were taken out of the bottles and the adhering fungus removed. The oven dry weights of the test blocks were then measured to determine the extent of decay.

Determination of moisture absorption by treated test blocks

Wood test blocks treated as above were steam sterilized and placed in culture bottles each containing moist soil and a feeder strip but no fungus. After 5 weeks, the moist test blocks were removed from the jars and immediately weighed. Comparisons of these weights with the respective oven dry weights were used to determine the extent of moisture absorption achievable under the moisture regime present in the soil bottles.

Isolation of resin acids from decayed rest blocks

After the decay studies, those test blocks which had originally been treated with the 100mM resin acid solutions were extracted (20 hr) with diethyl ether using a Soxhlet apparatus. After drying the ether extracts *in vacuo*, the resin acids were identified asbefore.

Results and Discussion

Identification of resin acids

Very little information is available regarding the nature of the extractives present in conifer seed cones. Among the extractives that have been identified are the resin acids which were characterized in the oleoresin exuded from cedar *(Cedrus libani)* cones and in ether extracts of selected pines (*P. jeffreyi, P. sylvestris, P. contorta, P. lambertiana)* (Haflzoglu and Holmbom 1987; Micales *et al.* 1994). In our research, the identity of the resin acids present in two pines (*P. banksiana and P. ponderosa)* and one spruce *(P. glauca)* were determined. For comparative purposes, the resin acids in the leaves, bark and wood of *P. banksiana* were also identified.

The resin acids were extracted with diethyl ether using a Soxhlet apparatus. Subsequent extractions with additional solvents of increasing polarity were then carried out to determine the total yields of non-volatile extractives in each tissue. The total yields of non-volatile extractives from the seed cones (9.7-15.3 %), determined as the weight percent of the dry tissue extracted, were intermediate of those ob-tained from the wood (4.0%) and bark (31.8-35.5%) samples; the greatest yield (45.1 %) of extractives was obtained from the leaves.

Among the woody tissues extracted *(i.e.* wood, bark, cones), the distribution of extractives obtained with the increasingly polar solvents indicated that the bark tissues contained a greater relative proportion of polar extractives than the wood. In contrast, the relative distribution of extractivs from the seed cones and wood were on average quite similar. However, in the seed cones, the amount of ether soluble extractives was typically 3-fold higher than that found in the wood. It remained to be determined whether the seed cones were unique in their resin acid compositions.

Ether extracts from each tissue were fractionated over DEAE-Sephadex to partially purify the resin acids which were then identified by gas-liquid chromatography of their methyl-ester derivatives. As shown in Table 1, the majority of the resin acids in each tissue were of the abietane type (see Fig. 1) as typically observed in the oleoresin of many pines (Zinkel 1981). For the seed cones, abietic, dehydroabietic and isopimaric acids comprised a significant proportion (63.7-80.5 %) of the total resin acids identified.

Analysis of the resin acids in the *P. glauca* cones revealed that dehydroabietic acid comprised as much as 56.6 % of the total resin acids identified. Other predominant resin acids in these seed cones were sandaracopimaric acid and hydroxyanticopalic acid. In the case of the *P. ponderosa* cones, a substantial amount (11.3 %) of imbricataloic acid was observed. This is a significant result since this resin acid has not been previously identified in the respective wood (Anderson *et al.* 1969; Conner *et al.* 1980a; Riffer and Anderson 1966) and therefore this is apparently

Table 1. Relative percents of resin acids in conifer seed cones, leaves, bark and wood

	Pinus ponderosa	*Picea glauca*	*Pinus" banksiana*				
	Seed cones	Seed cones	Seed cones	Leaves	Young bark	Old bark	Wood
Abietane type:							
Abietic acid	21.7	7.3	31.3	12.1	20.7	13.2	9.1
Dehydroabietic acid	26.9	56.6	16.3	55.4	31.9	48.4	29.7
Levopimaric acid	1.5	0.0	8.6	6.3	12.4	14.7	27.5
Neoabietic acid	1.7	0.0	17.6	3.4	5.0	2.0	3.4
Palustric acid	2.1	0.0	2.4	0.0	3.2	8.9	18.6
Pimarane type:							
Isopimaric acid	28.1	16.6	16.1	4.7	10.1	2.4	1.2
Pimaric acid	2.4	0.0	1.5	LO	10.1	7.3	8.1
Sandaracopimaric acid	3.9	12.1	3.0	8.8	3.5	2.7	2.2
Miscellaneous:							
Hydroxyanticopalic acid	0.0	6.8	1.0	0.0	0.0	0.0	0.0
Imbricataloic acid	11.3	0.0	2.0	8.3	3.1	0.4	0.2
Secodehydroabietic acid	0.4	0.6	,0.2	0.0	0.0	0. 0	0.0
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0

•Seed cones were collected in a young stand (-15 years old) of *Pillus ba11ksiana* from which one tree was felled to obtain leaf, bark and wood samples. Bark designated as young (1-2mm thick) was peeled from the upper few feet of the bole working downward whereas old bark (5-6mm thick) was peeled from the base of the bole working upward.

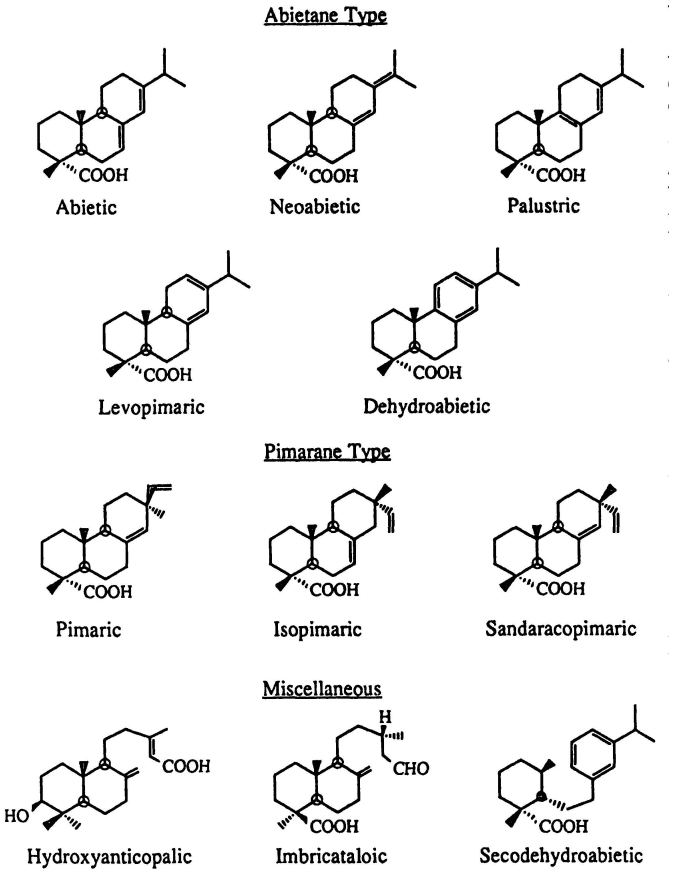

Fig. 1. Structures of common resin acids from *Pinus* species.

the first identification of irnbricataloic acid in P. **ponderosa.**

Analyses of the resin acids in the seed cones of P. **banksiana**in comparison with the resin acid composition of the leaves, bark and wood revealed many differences. In addition to abietic, dehydroabietic and isopimaric acids, the cones contained substantial amounts of levopimaric and neoabietic acids. The amount of neoabietic acid in the seed cones was more than 3-fold that in any of the other P. **banksiana** tissues. Comparing the distribution of resin acids in the seed cones with that obtained from the wood revealed higher amounts of abietic, neoabietic and isopimaric acids and lower amounts of levopimaric and palustric acids. These results are especially intriguing when compared with a literatur report of the resin acid composition of *P.* **banksiana**wood where levopimaric acid was found at very low levels of 1% or less (Conner **et al.** 1980b). One concern to us is that levopimaric acid readily undergoes isomerization to other resin acids depending upon the conditions under which the woody sample is handled (Soltes and Zinkel 1989). Prompt freeze-drying and storage under refrigeration may have allowed us to preserve the levopimaric acid normally present in the intact tree.

Upon comparison of the resin acids in young and old *P.* **banksiana**bark, a significantly greater amount of dehydroabietic acid was observed in the older tissues. This result was expected since oxidation coincides with shoot growth and maturation (Tobolski and Zinkel 1982).

Finally, of the tissues analyzed, the leaves have been shown to have the greatest seasonal variation in resin acid composition; the most stable resin acid composition is that present in the fall or winter (Tobolski and Zinke] 1982). We believe that our leaf samples, which were collected in early January, provide dependable data.

Decay inhibitionby resin acids

Screening experiments have shown that seed cone ether extracts, rich in resin acids, inhibit cultures of wood destroying fungi when incorporated into the agar growth medium (Micales **et al.** 1994). Incorabietane type was the most inhibitory; considerably less inhibition was observed for resin acids of the pimarane type. Such results suggest that the relative composition of resin acids may influence the rate of decomposition in a particular tissue.

Since softwoods are preferentially decayed by brown-rot fungi, we therefore decided to determine if the resin acids in softwoods contribute to their apparent resistance to decay by white-rot fungi. To test specific resin acids for their ability to inhibit these wood destroying fungi, we used a modified version of the ASTM (1992) wood test block method commonly used to test the efficacy of wood preservatives. This method, unlike screenings on agar media, simulates an environment conducive to decay whereby a natural substrate *(e.g.* wood) is gravimetrically monitored as it undergoes decomposition. In our research, hardwood (sweetgum) blocks, which are normally susceptible to decay by white-rot fungi, were impregnated with resin acids shown to be common amongst the conifer seed cones *(i.e.* abietic, dehydroabietic and isopimaric acids). Treated blocks and controls (with no resin acids) were then exposed to the white-rot fungi *J.* **lacteus** and *T.* **versicolor.**

As shown· in Table 2, untreated (control) hardwood blocks exposed to /. **lacteus** exhibited considerable decay as shown by an average weight loss 77.7%. Decreasing levels of decay were observed for those test blocks containing increasing retentions of either abietic or dehydroabietic acids. Likewise, increased resistance to decay was observed in test blocks treated with a 1 : 1 : 1 ratio of the 3 resin acids. In contrast, no significant difference in decay was observed for test specimens treated with isopimaric acid and the untreated controls which indicated that this particular resin acid was not especially inhibitory. This result

Table 2. Average weight loss of sweetgum test blocks exposed to while-rot fungi

Treatment	Concentration in solution	Retention (%)	Fungus	
			Jrpex /acteus (% [SD])	*Trametes versicolor* (% [SD])
Control	–	–	77.7 [2.0]"	53.3 [5.6]
Pentachloro-phenol	50mM	I.O[b]	0.3 (0.2]	3.1 [2.6]
Isopimaric acid	50mM	1.1	74.1 [3.8]	56.6 [3.4]
	IOOmM	2.2	77.5 [4.3]	54.0 [7.6]
	150mM	3.4	74.7 (3.3]	55.3 (4.2]
Abietic acid	50mM	I.I	71.8 [3.9]	50.3 [3.4]
	IOOmM	2.3	70.8 [2.3]	44.4 [3.7]
	150mM	3.4	60.5 [4.2]	40.6 [3.5]
Dehydroabietic acid	50mM	I.I	67.9 [4.9]	51.8 [3.0]
	IOOmM	2.3	64.8 (4.0]	51.5 [3.8]
	150mM	3.3	59.6 (2.2]	52.2 [2.9]
1: I : I Ratio	50mM	I.I	70.1 (4.9]	52.2 [4.2]
	IOOmM	2.3	65.6 (4.8]	49.5 [5.2]
	ISOmM	3.5	59.4 [4.2]	49.2 [3.7]

"Values expressed as percents of the starting dry weights of test blocks exposed to wood decay fungi after correcting for the increases in weight resulting from the added treatment compounds. For each treatment and fungus, there were 15 replicates. bValues expressed as weight percents of compounds in treated test blocks.

was anticipated since previous screenings of wood destroying fungi revealed little inhibition with pimarane resin acids (Micales *et al.* 1994).

In parallel experiments conducted with *T. versicolor,* isopimaric acid also did not impart any decay resistance at the concentrations used. This lack of decay inhibition for both fungi was intriguing since the apparently decay resistant seed cones contain significant amounts of isopimaric acid. Although relatively ineffective alone, synergism between isopimaric acid and other resin acids, or even distinctly different compounds, may be operative in seed cones to provide the natural durability. Alternatively, since resin acids have been suggested to be the adhesive that binds the scales together in seed cones (Kossuth and Biggs 1981), isopimaric acid may serve a critical physical role by contributing to the bonding and sealing of the cones.

Exposure of the other resin acid treated blocks to the fungus *T. versicolor* revealed significant decay inhibition only for those treatments utilizing abietic acid. Dehydroabietic acid was not inhibitory at the concentrations used. This result contrasts with previous screenings of this resin acid with *T. versicolor* on agar where inhibition was equivalent to that observed for abietic acid (Micales *et al.* 1994). Accordingly, dehydroabietic acid may exhibit less inhibition in a natural substrate than originally thought. In other experiments with *T. versicolor,* where concentrations used to treat soil blocks were 3-fold higher than those used in this investigation, inhibition by dehydroabietic acid was suggested (Hart *et al.* 1975). Unfortunately, because the purity of the dehydroabietic acid used in the Hart *et al.* study was low (*ca.* 80 %), the specific concentration of dehydroabietic acid necessary to obtain inhibition is yet uncertain.

Having established that specific resin acids exhibit varying capacities to inhibit the decay of a woody substrate, we turned our attention to the possible mechanism(s) employed. Generally, it has been surmised that oleoresin, produced by pines as a form of defense, is comprised of toxic volatile constituents in which the less toxic resin acids are dissolved. Once the volatile materials evaporate, a solid hydrophobic barrier is formed. Although presumably having limited toxicity to fungi, the resin acids could still serve an important role in decay resistance by lowering the tissue moisture content below fiber saturation thereby retarding fungal growth (Panshin and de Zeeuw 1970).

In order to determine the effects of the resin acids on wood moisture content, treated test blocks were placed in culture bottles prepared as before but without fungus. Maintained in parallel to the decay experiments, these test blocks were allowed to equilibrate with the moist atmosphere provided. At the end of the incubation period, the test specimens were immediately weighed, oven dried and weighed again to determine the amount of moisture present relative to the controls without resin acids.

For the control test blocks and those qea!ed with pentachlorophenol, nearly identical mos_tlire contents (*ca.* 57 %) were obtained. Accordingly, the low levels of decay for the test blocks treated with pentachlorophenol appear to rely solely on the extremely toxic nature of this compound. In contrast, the moisture contents of the test blocks treated with abietic acid and dehydroabietic acid were on average significantly lower at 42.6% and 47.4%, respectively. the blocks treated with the 1: 1: 1 mixture gave the lowest value for moisture content of 39.5 %. II). the test.. blocks treated with isopimaric acid, the only resin acid tested that did not impart any significant decay resistance, the average moisture content was 53.2 %. Thus, it appears that the observed differences in decay inhibition imparted by the different resin acids are related to the ability of each compound to form a hydrophobic barrier. The decay susceptibility of a given woody tissue (*e.g.* seed cones) would therefore be dependent upon the total amount of resin acids, and their specific composition, to provide the necessary hydrophobicity to lower the. moisture contc;vt to

levels near the fiber saturation point *(ca.* 30 %) below which fungal decay could cease altogether.

In addition to providing a barrier to decay through water repellency, resin acids may also be inherently decay resistant. Unfortunately, data regarding the ability of wood decay fungi to degrade resin acids is quite limited. Resin acids have been presumed to be decay resistant although some fungi *(Fomes pinicola* and *Haplosporella* sp.) have been reported to utilize levopimaric acid as a sole carbon source in the absence of carbohydrates (Shriner and Merrill 1970). Many wood decay fungi may be able to degrade resin acids but prefer other cell wall constituents *(e.g.* lignin, simple sugars, polysaccharides) when penetrating a woody substrate.

To determine if resin acid decomposition coincided with decay, resin acid treated test blocks, remaining after the decay studies, were extracted with diethyl ether. Resin acids present in the extracts were then identified by gas-liquid chromatography as before. Using the estimates for the original retentions of resin acids in the test blocks, weight percents of the resin acids recovered were calculated. As shown in Table 3, generally less than half of the resin acids incorporated into the respective test blocks were recovered when compared to the non-inoculated controls. Thus, it is apparent that resin acid degradation in a woody substrate by white-rot fungi is indeed significant.

At this juncture, it should be recalled that dehydroabietic acid incorporated into test blocks imparted decay resistance in experiments with */. lacteus* but not *T. versicolor.* Since resin acids appear to impart decay resistance by their water repellency, resistance to decay by both fungi would be expected as observed for test blocks treated with abietic acid and the 1:1:1 ratio mixture of resin acids. With fungal decay, it is quite likely that the moisture content of the test blocks would change, especially if the hydrophobic resin acids were undergoing degradation. In the case of the test blocks decayed by */. lacteus,* despite the high levels of decay (64.8-77.5 %), as much as 50 % of the resin acids were recovered. The high recovery of these amounts of resin acids suggests that this fungus preferentially degraded the woody substrate over the incorporated resin acids. In contrast, *T. versicolor* was apparently more efficient at degrading resin acids, such as dehydroabietic acid, as shown by the low resin acid recovery. Accordingly, we can account for the lack of decay resistance from dehydroabietic acid against *T. versicolor* due to the efficient degradation of the resin acid by this fungus.

From the data shown, it is quite apparent that not only the total amount of resin acids in a woody substrate, but also the relative proportion of the individual resin acids, can affect its susceptibility to decay. Moreover, it must be realized that although a particular resin acid *(e.g.* isopimaric acid) may be relatively inefficient alone, it may be a critical component of a resin acid mixture. As observed for the test blocks degraded by *T. versicolor,* the recovery of the resin acids was much greater in the 1: 1: 1 mixture of resin acids than that observed for each of the individual resin acid treatments. Likewise, abietic acid, a typically unstable resin acid, was recovered in a greater relative amount as a component of the resin acid mixture in the */. lacteus* decayed test blocks. Thus, although not necessarily efficient in resisting fungal decay, certain resin acids may serve a critical role in stabilizing other resin acids that are more effective in providing fungal decay resistance.

Table 3. Recovery of resin acids from sweetgum test blocks degraded by white-rot fungi

Treatment solution (100mM)	Fungus	
	Irpex lacteus (%)	*Trametes versicolor* (%)
Isopimaric acid	48.8[a]	30.9
Abietic acid	18.0	20.5
Dehydroabietic acid	50,1	11.2
1 : 1: 1 Ratio:		
Isopimaric acid	37.5	38.9
Abietic acid	39.0	42.5
Dehydroabietic acid	30.6	33.4

[a] values expressed as weight percents of resin acids originally incorporated into test blocks relative to the recovery of resin acids in control test blocks *(i.e.* treated *with* resin acids but not exposed to wood decay fungi).

Conclusion

Resin acids identified in conifer seed cones show varying capacities to inhibit decay by white-rot fungi. Experimental results indicate that resin acids may be inherently resistant to decomposition and thus protect a woody substrate from decay. In addition, they may inhibit decay by imparting water repellency. Although certain resin acids may not be efficient in inhibiting fungal decay, they may serve an important role in natural decay resistance by stabilizing those resin acids which are inhibitory.

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