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Efficacy of Pinosylvins against White-Rot and Brown-Rot Fungi

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Summary

Three stilbenes, pinosylvin (PS), pinosylvin monomethyl ether (PSM) and pinosylvin dimethyl ether (PSD), were extracted from white spruce (*Picea glauca*), jack pine (*Pinus banksiana*), and red pine (*Pinus resinosa*) pine cones, and their structures were confirmed by spectroscopic and chromatographic (HPLC, GC/MS, NMR and FTIR) analysis, PS, PSM, PSD or a 1:1:1 mixture of these stilbenes at concentrations of 0.1% and 1.0% were examined for their fungal inhibitory activity by two bioassay methods. Growth of white-tot fungi *(Trametes versicolor* and *Phanerochaete chrysosporium),* and brown-rot fungi *(Neolentinus lepideus, Gloeophyllum trabeum* and *Postia placenta)* on agar media in the presence of each of the stilbenes or a 1:1:1 mixture inhibited growth of white-rot fungi, but slightly stimulated growth of brown-rot fungi. Soil-block assays. conditions more representative of those found in nature. did not correlate with those from the screening on agar media. PS, PSM, PSD or a 1:1:1 mixture of the three compound\ at concentrations of 0.1 % and 1.0% did not impart any significant decay resistance to white-rot fungi inoculated on a hardwood (Red maple). However under the same conditions, decay reststance was observed against brown-rot fungi on a softwood (Southern yellow pine). It appears that stilbenes at least partially contribute to wood decay resistance against brown-rot fungi.

Introduction

Phenolic compounds are widely present in plants. and their presence often is associated with decay resistance (Hart and Hillis 1974; Hillis and Inoue 1968; Scheffer and Cowling 1966). Stilbenes, a class of phenolic compounds. are widely known as being fungitoxic and are important as growth inhibitors in lower plants (Valio *et al.* 1969; Adaskaveg 1992). In addition, these compounds have also been reported to be phytoalexins (Hart and Shrimpton 1979: Hanawa *et al.* 1992). Phytoalexins are synthesized in plants as a dynamic response to mechanical wounds and to injury by fungi or insects. They protect the plant from infection and subsequent decay (Loman 1969). The role of phytostilbenes in decay and disease resistance is well known (Hart 1981).

The mechanism of action of pinosylvin and its monomethyl ether derivatives has been studied by Lyr (1961) who showed that the toxic properties of stilbenes are based on the inactivation of fungal enzymes which contain -SH groups in their active sites. Formation of stilbenes occurs when tissues are dying slowly, usually due to desiccation of tissues (Jorgensen 1961). Stilbenes are also synthesized as a secondary response against mechanical wounds, fungal infections and insect infestations, and they complement the action of resin acids which also have fungitoxic properties (Prior 1976; Eberhardt *et al.* 1994).

The phytochemical composition of conifer seed cones from white spruce *(Picea glauca),* jack pine *(Pinus banksiana),* and red pine *(Pinus resinosa)* was examined in our laboratory. We have demonstrated that pine cones contain resin acids (Eberhardt *et al.* 1994) and tannins (Eberhardt and Young 1994) which have potential applications as preservative and antifungal agents. In this study, we undertake the characterization of different group of phenolic compounds present in pine cones. The purpose of this study is to examine the composition of stilbenes in pine cones and to evaluate the fungitoxicity of pinosylvin and its methyl ether derivatives on white-rot and brown-rot fungi using traditional bioassay media (2% malt extract agar) and soil-block bottle tests.

Experimental

General procedure

NMR spectra were measured in $(CD_3)_2CO$ employing a Bruker AC + 300 (^1H: 300 MHz: ^{13}C: 75 MHz) spectrometer. Mass spectra were obtained by GC/MS on an HP-5972 instrument (EI, 70 eV). Infrared spectra were recorded as NaCl or KBr discs on a Mattson Sirius 100/Cygnus 25 spectrometer. HPLC analysis and purification were carried out on a Beckman instrument; peaks were detected by UV at 280 and 305 nm and the column (250 × 4.6 mm) was an Altex ODS Serial #2UE271. Column chromatography was performed on silica gel (70 – 230 mesh); TLC was performed on silica gel (Kieselgel, 60F254) and the spots were visualized by UV (254nm) and by exposure to a solution of phosphomolybdic acid (5 % in ethanol). The statistical significance was determined using Dunnett's Method (ANOVA). The significant difference was that compared to the control.

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Plant material

Pine bark, cones, or sapwood of white spruce *(Picea glauca),* jack pine *(Pinus banksiana),* and red pine *(Pinus resinosa)* were collected in Wisconsin in 1997. The pines cones were washed and cleaned of all the solid residues with distilled water, dried at 40 °C for 24 hours, then ground in a Wiley mill (4 mm mesh size)

Extraction, isolation and identification of pinosylvins

The dry powdered pine cones were extracted with *n*-hexane for 24 hours using a Soxhlet apparatus. The hexane extracts were squentially extracted with 100% ether, acetone, methanol and water in increasing order of polarity.

The crude hexane, ether and acetone extracts were concentrated and subjected to chromatographic purification on a silica gel column using a n-hexane/ethyl acetate (2/1) system (Frankel *et al.* 1995; Rowe *et al.* 1969; Suga *et al.* 1993). Pinosylvin (PS) and pinosylvin monomethyl ether (PSM) were separated by repeated chromatography. PSM was methylated (CH_3I/K_2CO_3/acetone) and purified by open column chromatography to provide pinosylvin dimethyl ether (PSD) with a 96% yield.

Fungi

Three brown-rot fungi, *Neolentinus lepideus* (Fr.: Fr.) Redhead and Ginns (isolate MAD-534), *Gloephyllum trabeum* (Pers.: Fr.) Murrill (isolate MAD-617) and *Postia placenta* (Fr.) M. J. Larsen et Lomb. (isolate MAD-698), and two white-rot fungi, *Trametes versicolor* (L. Fr.) Pilát (isolate MAD-697) and *Phanerochaete chrysosporium* Burds. (isolate BKMP- 1767). were used in these experiments. The isolates were stored at 4 °C on 2 % malt-extract agar (MEA) (Difco).

Determination of antifungal activity on agar

For the growth study, the fungi were grown on MEA containing 0.1 and 1.0 mg/ml of each stilbene, PS, PSM, and PSD or a 1:1:1 mixture prepared from 0.1 mg/ml of each stilbene. The stilbenes were initially dissolved in ethanol (100%) and added to the MEA after autoclaving. The media were inoculated at the edge of the petri plate with white- or brown-rot fungi as mycelial plugs taken from cultures grown on MEA. The MEA without stilbenes was used as a control. The fungi were inoculated onto triplicate plates and were incubated at 27°C and 70% relative humidity. The antifungal activities were determined by measuring the radius of the fungal colony after subtracting the diameter of the inoculum plug after every two days for 8 days.

Determination of antifungal activity by soil-block bottle tests

Decay resistance imparted by stilbenes was also determined by a modified version of the ASTM (1992) procedure used to test the efficacy of wood preservatives. Softwood\ are preferentially decayed by brown-rot fungi, and hardwood\ by white-rot fungi (Zabel and Morrell 1992). To determine antifungal activity of stilbenes using soil-block assay, white- and brown-rot fungi were grown on pieces of a hardwood (Red maple) or a softwood (Southern yellow pine) (41 mm × 28mm × 3 mm the long dimension parallel to the grain) in bottles containing moist soil (silt-loam, the Bruce company, Middleton, WI). Each fungus was Inoculated into five replicate bottles and maintained in darkness at 27°C and 70 % relative humidity for three weeks.

Hardwood or softwood test specimens (6 mm × 6 mm × 3 mm), 5 blocks for each treatment, were dried (103 2°C). weighed and impregnated by vacuum with a 0.1 % and 1.0% solution (w/v) of each stilbene or a 1:1:1 mixture of these stilbenes in ethanol. The wood specimens were treated with ethanol as a control. The treated test specimens were placed on the top of the maple or pine blocks that had been inoculated previously with white- or brown-rot fungi. The test blocks were incubated in darkness at 27°C and 70% relative humidity for six weeks.

At the end of the exposure, the test blocks were cleaned of fungal residue, dried (24 hours at room temperature, then at 80°C over night), and then weighed. The decay reststance of the different fungi was determined by the loss of weight of the treated test specimens compared to the untreated wood blocks (controls).

To determine if the extracted stilbenes influenced the moisture regime on wood and then affected fungal attack, the wood moisture content was determined using treated test specimens without fungal treatment. Maple and Southern yellow pine test specimens were oven-dried for 2 hours and weighed. Thereafter, the test specimens were impregnated with 0.1% and 1.0% of each stilbenes or a 1:1:1 mixture of the three stilbenes. Hardwood and softwood test specimens were also treated with ethanol as a control. The test specimens were equilibrated at room temperature for 3 hours, oven-dried and weighed

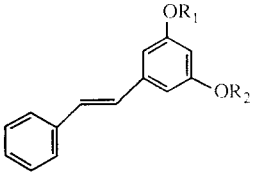
Results and Discussion

Identification and quantification of pinosylvins

Analysis of the hexane, ether and acetone extracts of the dry pine material by TLC revealed fluorescent peaks under 254 nm and 360 nm UV light. characteristic of polyphenolic compounds (Langcake and Pryce 1976). The pine extracts were identified by HPLC, GC/MS, FTIR, ^{1}H- and ^{13}C-NMR spectral as PS, PSM and PSD (Fig. I) (Frankel *et al.* 1995; Rowe *et al.* 1969; Suga *et al.* 1993). Their structures have also been characterized on the basis of the GC/MS spectra of the corresponding acetate by comparison with GC/MS data reported in the literature (Lin *et al.* 1992; Geraldo *et al.* 1996). The hexane, ether and acetone extracts were purified by repealed open column chromatography and the isolated stilbenes were used in quantification and antifungal studies. PSD, which was not easily isolated from the extract, was obtained by methylation of PSM.

The stilbene composition of cones, bark, and wood was determined for three softwood species, white spruce *(Picea glauca),* jack pine *(Pinus banksiana),* and red pine *(Pinus resinosa).* The total amount of non-volatile extracts from bark, cones and wood was determined as the weight percent of the dry material extracted. The yields of extractives from bark (43-54 %) was higher than that from seed cones and wood (28-34 % and 14-23 %, respectively).

Table I shows the percentage of stilbenes in the acetone extracts from tissues of the different species carried out by GC/MS and HPLC. The percentage of stilbenes was different in all tissues from the various species. PSM was the most abundant stilbene in the plant species evaluated; it represented 5.5.4-70.9% of the total stilbenes. PSD was the least concentrated in all tissues and all species, representing 5.0-9.3% of total stilbenes. and was only detectable by GC/MS and HPLC.



PS : $R_1=R_2=H$
PSM: $R_1=H$, $R_2=Me$
PSD : $R_1=R_2=Me$

Fig. 1. Structure of pinosylvins.

Bioassays with pinosylvins

The growth or fungi in the bioassay media was used as a means of evaluating the fungitoxicity properties of the isolaled stilbenes. Preliminary experiments were conducted *in vitro* on agar media with the extracted stilbenes to assess the antifungal activity of the crude preparations (Micales *et al.* 1994). The crude preparations that showed antifungal activity were further purified by open column chromatography to isolate and identify the active components.

PS and PSM isolated from the crude extract of white spruce and pine bark, cones and wood, and PSD were screened with the agar media assay to elucidate their antifungal activity. Table 2 shows that the white-rot fungi. *T. versicolor* and *P.* chrysosporium, were inhibited by the three stilbenes tested. In fact, PS, PSM and PSD were all effective in reducing growth of *P. chrysosporium* at both concentrations. Mixing the pinosylvins in a 1:1:1 ratio increased the efficacy against white-rot fungi. The most effective stilbene was PSD on the growth of of *P. chrysosporium* which resulted in a growth reduction of 28 % at 0.1% PSD and 32% at 1.0% PSD (w/v) compared to the untreated control. The inhibitory effects of PS were less effective than PSD; the growth of *P. chrysosporium* was decreased by only 18% and 23% at concentrations of 0.1% and 1.0%, respectively. The 1:1:1 mixture appeared to inhibit the growth of this fungus slightly at 0.1% (7.0 %). but the reduction of growth was much greater at the 1.0% level (30 %), *T. versicolor* was also inhibited signficantly by PS and PSM ($0.001 < P < 0.05$), but not by PSD or the 1:1:1 stilbene mixture.

To determine a possible broader efficacy of the stilbenes, we also examined the antifungal activity of these compounds using three brown-rot fungi. Growth of the brown-rot fungi. *N. lepideus, G. trabeum* and *P. placenta,* was only slightly affected by the treatment with stilbenes. PS and PSM displayed slight fungitoxicity against *N. lepideus* at concentrations of 0.1% and 1.0% (Table 2). The average

Table 1. Relative percentage of stilbenes in bark, cones and wood from three plant species*

	Picea glauca			*Pinus banksiana*			*Pinus resinosa*		
	Bark	Cones	Wood	Bark	Cones	Wood	Bark	Cones	Wood
Pinosylvin (PS)	21.6	33.1	30.8	30.9	31.7	20.5	34.2	30.5	35.3
Pinosylvin monomethyl ether (PSM)	70.9	60.2	59.5	61.0	67.5	70.7	58.2	64.5	55.4
Pinosylvin dimethyl ether (PSD)	7.5	6.7	9.7	8.1	5.8	8.8	7.5	5.0	9.3

* The relative percent of each stilbenes was determined as the weight percent of the dry material extracted; all totals are 100%.

Table 2. Percent Inhibition of the radial growth of white-rot fungi (4 days) and brown-rot fungi (6 days) inoculated on malt-agar media containing various concentrations of stilbenes

		Percentage inhibition[a]				
		White-rot fungi		Brown-rot fungi		
	Treating solution % (w/v)	*Trametes versicolor*	*Phanerochaete chrysosporium*	*Neolentinus lepideus*	*Gloeophyllum trabeum*	*Postia placenta*
Pinosylvin (PS)	0.1	*2.9	*18.3	4.4	*– 5.1	*7.7
	1.0	*11.1	*23.1	2.9	*1.7	*11.5
Pinosylvin monomethyl ether (PSM)	0.1	*8.2	*8.7	3.0	*– 1.7	*12.5
	1.0	*20.5	*32.0	6.0	*– 11.5	*13.9
Pinosylvin dimethyl ether (PSD)	0.1	0.9	*28.3	*1.4	*5.1	11.5
	1.0	5.4	*32.2	*2.9	*11.9	19.2
1:1:1 Mixture	0.1	– 1.4	*6.7	– 1.4	0.0	*5.1
	1.0	9.9	*30.3	5.9	*– 5.1	*9.0

Significant difference *($0.001 < P < 0.005$). Values expressed as an average of three replications, a Negative values: % stimulation.

growth inhibition was 4.4% which was not significantly greater than the control (P > 0.2). PSD did exhibit a significant growth reduction on *N. lepideus* (1.4%), and the effect was slightly increased at the 1.0% level (2.9%, P = 0.049). The 1:1:1 mixture of the stilbenes did not affect growth of *N. lepideus* at either of the concentrations. PSM and the 1:1:1 stilbene mixture stimulated the growth of *G. trabeum* at both concentrations. Growth of *G. trabeum* in the presence of PS was stimulated at a concentration of 0.1%, which was significantly different from the growth at 1.0%, which was slightly inhibited (P = 0.018). PSD showed a highest toxic activity against this fungus at higher concentration (12% inhibition, P = 0.032).

The three stilbenes and the 1:1:1 mixture of these compounds showed the highest toxic effect against *P. placenta.* The concentration of the stilbenes determined the percentage inhibition. Although PSD decreased the growth of *P. placenta* by 11.5% at 0.1% concentration and 19.2% at 1.0% concentration. this difference was not statistically significant (P > 0.2). PS, PSM and the mixture of these compounds showed a statistically significant growth inhibitory effect on *P. placenta.*

These data show that stilbenes were more toxic to white-rot fungi than brown-rot fungi using the agar media assay. The toxic effect of stilbenes on the three brown-rot fungi was variable with the fungal species used. The concentration of the stilbenes also showed a variable influence on the white-and brown-rot fungal inhibition. Furthermore, growth inhibition of the white- and brown-rot fungi was not improved by using the 1:1:1 stilbene mixture.

To further confirm the agar assay data and to examine conditions more representative of those found in nature, we evaluated the antifungal activity of PS, PSM and PSD using the soil-block bottle assay. The decay resistance of a hardwood and a softwood species against white-and brown-rot fungi was tested with these stilbenes to determine their preservative properties. As shown in Table 3, control blocks exposed to the white-rot fungi, *T. versicolor* and *P. chrysosporium,* showed weight losses of 50.9% and 30.3% respectively. No increase in decay resistance was observed when the maple test blocks were treated with stilbenes or a 1:1:1 stilbene mixture at a concentration 0.1%. Only PSD significantly inhibited decay by *T. versicolor* at 1.0% (43.6% P = 0.002). Further, no significant improvement of decay resistance was observed in the maple block test with a 1:1:1 mixture of the three stilbenes. Thus. these stilbenes did not seem to inhibit decay by white-rot fungi, but instead appeared to stimulate wood decay.

Similar experiments were carried out on pine blocks inoculated with brown-rot fungi. Table 3 shows the toxic effect obtained when the wood test blocks were impregnated with the stilbenes or the 1:1:1 mixture at the 0.1% and 1.0% concentrations. The decay of unimpregnated pine control blocks, inoculated with *N. lepideus,* was 23.3%, whereas decay of wood by *G. trabeum* and *P. placenta* was 37.9% and 45.3%, respectively.

Decay resistance was slightly increased when softwood test blocks exposed to *N. lepideus* were treated with the three stilbenes and the 1:1:1 stilbene mixture. PS, PSM, and PSD inhibited decay significantly at both concentrations. However, the difference observed with the 1:1:1 stilbene mixture was not statistically significant. PS displayed the best fungitoxicity of the three stilbenes, reducing weight loss to 14–1.5% (P < 0.012).

PSD did not show any significant fungitoxicity against *G. trabeum* at either concentration. PS and the 1:1:1 mix-

Table 3. Decay of hardwood (Red maple) or softwood (southern yellow pine) samples treated with pinosylvins by white-rot and brown-rot fungi

			Percentage mass loss[a]				
			white-rot fungi		Brown-rot fungi		
	Treating solution % (w/v)	Retention (g/kg)	*Trametes versicolor*	*Phanerochaete chrysosporium*	*Neolentinus lepideus*	*Gloeophyllum trabeum*	*Postia placenta*
Control			50.9(2.2)	30.3 (6.0)	23.3 (4.0)	37.9 (2.2)	45.3 (5.8)
Pinosylvin (PS)	0.1	0.022	56.0 (5.1)	*60.7 (9.8)	*14.8 (0.9)	*29.8 (1.0)	*49.8 (3.6)
	1.0	0.21	56.7 (1.1)	*29.4 (4.2)	*14.0 (0.9)	*37.2 (3.5)	*29.5 (2.1)
Pinosylvin monomethyl ether (PSM)	0.1	0.022	*51.8 (5.0)	*54.4 (6.7)	*16.5 (2.0)	*43.8 (8.3)	43.4 (5.6)
	1.0	0.23	*58.9 (2.4)	*39.5 (9.9)	*20.5 (1.4)	*17.5 (4.0)	39.3 (3.7)
Pinosylvin dimethyl ether (PSD)	0.1	0.022	*57.0 (6.2)	33.1 (7.8)	*18.4 (0.7)	38.1 (4.4)	*36.6 (3.2)
	1.0	0.21	*43.6 (2.9)	40.8 (6.3)	*16.4 (1.8)	36.9 (3.3)	*26.5 (0.8)
1:1:1 Mixture	0.1	0.021	54.4 (5.5)	*44.8 (8.7)	20.9 (2.4)	*31.0 (2.0)	41.3 (1.6)
	1.0	0.20	58.8 (3.8)	*41.7 (8.7)	23.2 (3.1)	*23.3 (1.4)	41.1 (5.5)

[a]Value expressed as percent of the starting dry weights of test blocks exposed to wood decay fungi after correcting for the increase weight resulting from the added treatment compounds. For each treatment and fungus, there were 5 replicates. Figures in parenthesis refer to the standard deviation. Significant difference *(0.001 < P < 0.05).

ture at 0.1 % and 1.0% concentration statistically reduced decay by *G. trabeum* (P = 0.01). PSM was able to partially suppress decay only at a concentration of 1.0% (w/v) (17.5 % mass loss, P = 0.01) (Table 3), but at the 0.1 % concentration, wood decay was increased significantly by *G. trabeum* (43.8%, P = 0.001).

Evaluation of the ability of *P. placenta* to decay wood in the presence of PS showed that the low concentration of PS did not impart any resistance to the softwood blocks, but instead produced a slight stimulation in decay (weight loss of 49.8 %) (Table 3). In contrast, a higher concentration of PS inhibited pine wood decay by *P. placenta* (29.5%, P = 0.001). The toxic effect of PSM on *P. placenta* was not statistically significant at either concentration (P > 0.4). PSD at both levels exhibited a significant suppressive effect on *P. placenta* with a weight losses between 26.5-36.6% (P = 0.001). The toxicity of the 1:1:1 stilbene mixture at both dosages (41.2 %, P > 0.38) was less pronounced than that observed with the individual stilbenes.

The results of the agar media evaluation support the hypothesis proposed in the introduction. that stilbenes are involved in the decay resistance of plants. Characterization of the antifungal properties of PS, PSM and PSD was carried out on agar media, which provides useful information about the potential toxicity of those compounds. Using 2% MEA, PS, PSM, PSD and a 1:1:1 mixture of these stilbenes, significant toxicity against white-rot fungi was exhibited but there was only a slightly toxic effect against brown-rot fungi. However, when the fungitoxicity of these stilbenes was tested in impregnated wood blocks, the decay resistance results did not correlate with those obtained in agar media assay. The toxic effect of PS, PSM, PSD and the 1:1:1 mixture were more pronounced on brown-rot fungi than on white-rot fungi with the soil-block test.

It should be pointed out that agar media results cannot be extrapolated to conditions found in nature. Indeed, agar media contain substances that are not present in *in vivo* conditions and can modify the behavior of the fungi. In *in vitro* bioassays, the stilbenes are free to interact with the fungi, whereas *in vivo,* they are bound to many different organic compounds. For instance, their *in vivo* production is bound to the synthesis of resin acids to protect the cells against desiccation and are not the only compounds responsible for decay-resistance (Hillis and Inoue 1968; Eberhardt *et al.* 1994; Hart and Hillis 1974; Jorgensen 1961).

After treatment, hardwood and softwood control test blocks had a moisture content of 57.4% and 58.0%, respectively (Table 4). Hardwood and softwood test blocks treatcd with PS at 0.1% concentration and the 1:1:1 mixture at both concentrations showed comparable moisture contents of 44% and 45.1%. Hardwood and softwood test blocks treated with PSM and PSD also had an average moisture content of 48%. All the stilbenes tested seem to protect the wood by their water repellency properties. In fact, these compounds are not water soluble and they can form a hydrophobic barrier to lower the moisture content of wood. Therefore, the fungi may have had little access to free water in the cells despite a moisture content level that should facilitate decay. The stilbenes seem to have a dehydrating effect on wood. which indirectly prevents wood decay.

Table 4. Moisture content of hardwood and softwood samples after impregnation with pinosylvins

	Treating solution % (w/v)	Moisture (%) Hardwood (Red maple)	Softwood (southern yellow pine)
Control		57.4	58.0
Pinosylvin (PS)	0.1	*44.8	*45.8
	1.0	44.6	*45.8
Pinosylvin monomethyl ether (PSM)	0.1	*47.9	*47.6
	1.0	47.9	47.7
Pinosylvin dimethyl ether (PSD)	0.1	*48.4	48.3
	1.0	*48.5	*48.3
1:1:1 Mixture	0.1	*44.1	*45.1
	1.0	*44.1	*45.1

Significant difference *(0.001 < P < 0.08).

Lyr (1961) and Scheffer and Cowling (1966) have investigated the toxic effect of stilbenes on the respiration of fungi. From these experiments the researchers proposed that the fungitoxic properties of the stilbenes are based on the inactivation of fungal enzymes that contain -SH groups on their active sites. In these studies, it was determined that enzymes associated with decay, such as laccase or pectinase, were less activated by -SH groups and therefore were not inhibited by stilbenes (Lyr 1961; 1962). White-rot fungi produce laccase, and they were not inhibited by stilbenes at high concentrations on agar media assays and soil block bottle tests. Brown-rot fungi do not produce laccase and were inhibited by stilbenes in both bioassay tests (Rudman 1962; Hart 1974; Loman 1969, 1970). The initial stages of brown-trot fungi decay involve oxidative degradation (Highley and Dashek 1998). Numerous surveys have shown that stilbenes have antioxidant properties (Gehm *et al.* 1997; Cai *et al.* 1997). PS, PSM and PSD may inhibit metabolites such as radical scavengers synthesized by brown-rot fungi and increase decay resistance against brown-rot fungi.

The concentration of PSD was lower than PS and PSM in all tissues from pine and spruce (Table I) and is not considered a toxic phenol (Hart and Shrimpton 1979), but this compound exhibited the best decay resistance against *P. placenta* at 0.1% and 1.0% (w/v). PS and PSM are present in a higher levels in all pine and spruce tissues and also exhibited decay resistance. PS is usually considered to be more toxic than PSM at low concentrations (Hart and Shrimpton 1979) which is confirmed here for the brown-rot fungi *N. lepideus* and *G. trabeum* (Tables 2 and 3). Overall. high concentrations of stilbenes did not positively affect the inhibition of decay by both white- and brown-rot fungi. In some cases, high concentrations stimulated the growth and

decay by these fungi, with PS having the highest antifungal activity at the low concentration.

This study has demonstrated that extracts from conifer seed cones contain stilbenes that may exhibit antifungal activity against white- and brown-rot fungi. The antifungal activity varied with the stilbene, the concentration, the fungi and the bioassay test used.

By impregnated wood tests. the results obtained correlated with those found in literature (Rudman 1961-1966; Valio 1969; Hart 1981; Hart and Hillis 1974: Highley and Dashek 1998; Loman 1969; Hart and Shrimpton 1979; Rennerfelt and Nacht 1955; Schultz *et al.* 1997). Brown-rot fungi were inhibited by PS, PSM, PSD and the 1:1:1 stilbene mixture. Because stilbenes are synthesized as a secondary response to adverse conditions. they may help to protect pine tissue and prevent the fungi from having access to free water in the cells. Inhihition of white-rot fungal degradation of wood blocks was not observed which demonstrates the importance of employing several analytical methods in the determination of fungal toxicity. Although these compounds are somewhat toxic against brown-rot fungi, they are not efficacious for wood preservation

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