

ABILITY OF NATURAL EXTRACTS TO LIMIT MOLD GROWTH ON DOUGLAS-FIR SAPWOOD

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The ability of selected plant extracts from wood and foliage to inhibit mold regrowth on fungal colonized wood was evaluated on Douglas-fir sapwood. Most foliage extracts produced some inhibition of *Graphium* or *Trichoderma* species, but isolations of other fungi increased following treatment. Five out of eight wood extracts produced 50% reductions in isolations, and those from Alaska cedar, western juniper, and incense cedar produced at least 80% reductions. The results indicate that wood extracts may be useful for reducing the incidence of mold on wood products, but none of the materials evaluated completely inhibited the test fungi. These extracts may provide a useful value-added application for by-products of lumber production from these species.

Keywords: Mold; *Graphium* spp.; *Trichoderma* spp.; *Aspergillus* spp.; *Penicillium* spp. leaf extracts; Xylem extracts; Douglas-fir sapwood

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INTRODUCTION

The presence of pigments, fungal spores, and hyphae on wood surfaces was formerly considered a nuisance by wood users, because of the negative visual impact (Scheffer 1973; Zabel and Morrell 1992). A number of high profile lawsuits concerning alleged health effects from mold in buildings have substantially altered this perception (Fung *et al.* 1998; Hardin *et al.* 2003; Kelman *et al.* 2004; Robbins and Morrell 2006; Robbins *et al.* 2000). While there remains a spirited debate about the risks of molds, builders and owners are faced with the prospect of having to limit their liability by minimizing mold growth and, when it does occur, identify safe methods for its elimination.

The most commonly recommended method for mold removal from lumber has been to wash the boards with dilute solutions of household bleach or soapy water (Robbins and Morrell 2006). Bleach solutions have long been used for removing biological contaminants from a variety of surfaces and they have the advantage of simultaneously brightening the wood surface. Bleach, however, does not completely eliminate surface fungi, and these fungi will rapidly recolonize the substrate under proper moisture and temperature regimes (Taylor *et al.* 2004). Thus, drying below 20%, the generally accepted minimum wood moisture level for fungal growth, remains an essential part of mold mitigation.

In some instances, it is difficult to achieve rapid drying or there is a long-term risk of rewetting. The application of fungicides can help alleviate this risk, but there are a limited range of biocides that can be safely used in inhabited structures. The primary chemical advocated for this purpose has been boron, which has relatively low toxicity to

humans, is easily dispersed in water, and has some long term benefits for limiting colonization by insects and decay fungi. Boron, however, has limited activity against many mold and stain fungi, and tests of several boron formulations showed that these systems were unable to either eliminate established fungi or prevent regrowth of fungi on Douglas-fir sapwood (Taylor *et al.* 2004). While there are a number of more robust chemicals that can limit growth of a broader range of fungi, a general public fear of chemicals largely limits their use in inhabited structures.

One alternative to the use of traditional biocides is the use of natural plant extracts. A variety of plant extracts have been shown to inhibit growth of a range of organisms, including many fungi (Ahn *et al.* 1998; Du *et al.* 2011; Li *et al.* 2008; Manter *et al.* 2006). These materials, although potentially as toxic as synthetic biocides, are more readily accepted by the general public. Among the many candidates for mold prevention are a group of extracts from the foliage and xylem of various wood species. The heartwoods of many species contain a diverse array of potentially inhibitory compounds (Hillis 1987), but there has been little work on the efficacy of these compounds against mold and stain fungi. Similarly, foliage of these same species can also contain bioactive compounds. These materials might be economically collected during normal harvesting and processing operations. In this report, we examine the ability of selected foliage and wood extracts to limit fungal growth on heavily colonized Douglas-fir sapwood pieces.

EXPERIMENTAL

Since the goal of this test was to evaluate the ability of extracts to limit regrowth of a range of fungi, the materials evaluated were not inoculated with any single fungus. Instead, the fungi present prior to chemical application were determined and then the ability of each extract to eliminate these same fungi at the end of the test was determined by reisolation. Although this created some anomalies when fungi were not always consistently isolated, it presented a more realistic control challenge. The ideal chemical would produce 100% reductions in isolations of all fungi.

Douglas-fir sapwood (*Pseudotsuga menziesii* (Mirb.) Franco) boards (19 by 137.5 mm by 2.4 m long) were obtained from a local lumber yard. Sapwood of this species is very susceptible to fungal attack, and the presence of mold on commercial lumber is a major concern for retailers. The material had been stored wet without chemical protection and was heavily colonized by a range of fungi. The lumber was then cut into a series of 25 by 25 by 50 mm long blocks that were randomly allocated to groups for testing. The fungal flora on the blocks prior to treatment was assessed by gently pressing one face of each wood sample onto 0.5% malt extract agar (MEA) (containing 0.7% streptomycin) in petri dishes to transfer any fungal spores as well as hyphal fragments present on the wood surface to the agar surface. The plates were incubated at room temperature (in darkness) and observed for evidence of fungal growth. Fungi were classified to genus either by direct examination of prepared slides under a microscope or by transferring to another petri dish containing MEA for later identification. Genera of particular interest for our study were *Paecilomyces*, *Trichoderma*, *Penicillium*, *Aspergillus*, and *Graphium*. The presence of *Sporothrix* spp. was also noted, since many members of this genus are anamorphs of *Ophiostoma* species. The former four genera are common molds on a variety of substrates, while *Graphium* is another imperfect state of *Ophiostoma*, a genus containing main common wood staining fungi (Kang and Morrell 2000). All genera have

been previously isolated from freshly sawn Douglas-fir sapwood (Kang and Morrell 2000). We also noted the presence of yeasts and dematiaceous hyphomycetes, although there was no consistent pattern in isolation of these fungi and these data will not be reported here. The presence of a given genus from an individual wood sample was counted only once, regardless of the number of colonies that grew. While we recognize that species in the same genera can have markedly different properties, our intent was to assess the overall effect of a given treatment on fungal growth using the genera as indicators, not to assess the effects on all possible species present.

Table 1. Effect of 0.1 mg/mL of Extracts of Leaves or Heartwoods of Various Tree Species on Ability to Isolate Selected Fungal Genera from Douglas-fir Sapwood Lumber 4 weeks after Treatment

Treatments	Method ¹	Re-isolation Frequency (%) ²					
		<i>Graphium</i>	<i>Trichoderma</i>	<i>Penicillium</i>	<i>Aspergillus</i>	<i>Paecilomyces</i>	<i>Sporothrix</i>
Control		25	100	100	25	50	25
Foliage Extract							
Douglas-fir	SD	50	X	+25	25	X	X
Incense cedar	SD	50	100	75	X	+25	+100
Alaska cedar	SD	75	100	X	50	X	X
Engelmann spruce	SD	100	100	+25	X	+50	X
Pacific silver fir	SD	100	100	X	25	+25	X
Ponderosa pine	SD	50	100	25	X	X	+50
Giant sequoia	SD	100	100	+25	75	+100	+100
Western white pine	SD	75	100	+25	100	+100	+25
Mountain hemlock	SD	75	50	25	75	+25	+25
Noble fir	SD	50	100	+25	75	+50	X
Grand-fir	SD	75	100	+25	25	+25	+25
Engelmann spruce	M	50	100	+50	50	+25	+25
Wood Extracts							
Alaska cedar	ETAC	50	100	25	X	X	X
Alaska cedar	SD	100	100	+25	X	X	X
Alaska cedar	M	X	100	100	50	X	X
Western juniper	SD	75	100	100	X	X	X
Western juniper	M	75	100	+75	100	+50	X
Incense cedar	SD	75	100	25	100	X	+100
Port Orford cedar	SD	50	75	50	X	+25	X
Commercial Samples							
Lemon oil	Comm.	X	100	100	25	X	X
Nootkatone	Comm.	X	100	X	X	+25	X
Peppermint	Comm.	50	100	X	100	+50	X

¹Where extraction methods were steam distillation (SD), methanol extraction (M), ethyl acetate extraction (EtAc), or commercial sources.

²Values represent means of 4 isolations per extract concentration. Those with a (+) value indicate that isolation frequency of this fungus increased while those with an X indicate that this genus was not isolated from the samples either before or after treatment on the same sample boards.

Groups of four blocks each were treated with aqueous solutions containing 0.1 mg/mL of extract that had been collected by either steam distillation (SD), methanol extraction (M), or ethyl acetate extraction (Et Ac). Additional extracts were obtained from commercial sources (Table 1).

Extracts were dissolved in ethanol prior to use, then appropriate amounts of the stock solution were added to water to produce the desired dilution. The concentration of ethanol in water varied from 0.3% to 3 % (V/V) depending on the amount needed to disperse the extract. Control solutions contained 3% ethanol.

Each block was dipped for 30 seconds into its respective extract, allowed to drain, and then placed in a plastic bag in a conditioning room for 4 weeks. In the first test, the blocks were incubated at 32 °C and 90% relative humidity (RH) for 1 week, then 23 °C and 65% RH for 3 weeks. The most promising extracts were further evaluated at 0.01, 0.05, or 0.1 mg/mL of extract using the procedures described above, except that the blocks were incubated at 23 °C and 65% RH for the entire test. The incubation period created conditions that allowed fungi propagules still present on or in the wood to resume growth. These conditions would represent an environment where treatment was applied but no attempt was made to control the moisture source to allow the wood to dry.

The degree of fungal colonization after treatment and incubation was assessed by pressing the same wood surface on 0.5% malt extract agar (containing streptomycin 0.7%). The degree of extract effect on fungal frequency was categorized as negative (*i.e.* isolations of a genus declined) or stimulatory (isolations increased after treatment). The incidence of a given genus was categorized on the basis of percent reisolation. In some instances, isolation frequency actually increased by 25 to 100% after treatment.

RESULTS AND DISCUSSION

Most leaf oil extracts were associated with reductions in isolations of both *Graphium* and *Trichoderma* species (Tables 1, 2).

Graphium is a common invader of freshly cut Douglas-fir sapwood, but its incidence tends to decline with time after harvest (Kang and Morrell 2000). It is possible that the extracts hastened this process. Isolations of *Aspergillus* spp. also declined with leaf oil treatments, but the effects were somewhat reduced compared to the impacts on *Graphium* or *Trichoderma* spp. Isolations of *Penicillium*, *Paecilomyces*, and *Sporothrix* spp. all increased following leaf oil treatment, suggesting that the chemicals modified the wood surface to encourage growth of these genera. Many *Sporothrix* species are states of *Ophiostoma* as are the *Graphium*. Thus, increased isolation of this genus may reflect changing environmental conditions rather than colonization by new species.

Of the 12 leaf extracts tested in the first trial, Alaska cedar and Pacific silver-fir leaf oils produced marked reductions in isolations, while the remainder had little effect, suggesting that they were not effective at the levels tested.

Wood extracts also produced sharp declines in isolation frequencies of *Graphium* and *Trichoderma* species. *Aspergillus* species, where present in the original controls, were also reisolated at lower levels in many treatments. Unlike the leaf oils, wood extracts were not associated with sharp increases in isolations of *Penicillium* or *Paecilomyces*. Furthermore, five of the eight extracts tested produced substantial reductions in isolations and three (Alaska cedar (M), western juniper (SD), and incense cedar (SD)) produced nearly complete reductions in isolation. Clearly, these extracts had some potential for limiting mold activity.

In the second trial, extracts were tested at a range of dilutions (0.01 to 0.10 %) to determine a minimum effect level for each system. Commercially obtained lemon oil, which performed well in the first trial, was included as a comparative treatment.

Table 2. Effect of Treatment with Concentrations of Extracts from Leaves or Heartwood Extracts from Various Tree Species on Ability to Isolate Selected Fungal Genera from Douglas-Fir Sapwood Four Weeks after Treatment ²

Treatment	Conc (mg/ml) ¹	Extraction	Reisolation Frequency (%) ²					
			<i>Graphium</i>	<i>Trichoderma</i>	<i>Penicillium</i>	<i>Aspergillus</i>	<i>Paecilomyces</i>	<i>Sporothrix</i>
Control		-	X	100	X	25	25	X
Lemon oil	0.01	Comm.	25	25	50	25	X	X
	0.05		100	100	25	25	X	X
	0.10		75	50	100	X	X	+25
Western juniper	0.01	SD	X	100	50	25	100	+50
	0.05		X	100	75	50	100	X
	0.10		X	100	25	75	100	+25
Cedar HW	0.01	SD	+25	100	+25	100	75	25
	0.05		X	75	25	50	50	X
	0.10		25	100	X	25	100	X
Alaska cedar HW	0.01	SD	75	75	X	25	X	+25
	0.05		100	100	100	25	X	X
	0.10		100	100	75	25	X	+25
Douglas-fir leaf oil	0.01	SD	X	100	X	50	X	X
	0.05		50	100	X	25	X	X
	0.10		50	100	100	50	X	+50
Incense cedar leaf oil	0.01	SD	25	100	50	50	100	+50
	0.05		X	100	50	X	25	25
	0.10		X	50	X	75	100	25
Incense cedar wood	0.05	SD	25	100	50	X	100	X
	0.10		X	100	75	75	75	+25

¹Methods of extraction including steam distillation (SD) and commercial sources (Comm).

²Values represent means of 4 isolations per extract concentration. Those with a (+) value indicate that isolation frequency of this fungus increased while those with an X indicate that this genus was not isolated from the samples either before or after treatment on the same sample boards

In principle, one would expect increasing extract concentrations to produce a corresponding reduction in fungal isolations; however, there was no consistent relationship between treatment level and isolation. Untreated blocks exposed to the same conditions as the treated samples experienced a slight decline in isolations, suggesting that small reductions in isolation frequency were associated with declines in spore viability over time rather than any chemical effect.

Lemon oil produced general reductions in isolations of all genera except *Sporothrix* spp. at the 0.05 and 0.10% dilution levels. All of the wood extracts produced reductions in isolations that exceeded the changes in control samples, although the lack of a dose response suggests that further increases in extract concentration might not noticeably improve treatment. *Trichoderma* species appeared to be the most sensitive genus to the extracts, while *Penicillium* and *Paecilomyces* species appeared to be more variable in their response. Isolations of *Graphium* species also declined in many cases. The increased

isolation of *Sporothrix* species suggests that the extracts may have altered the physiology of the fungus rather than completely inhibiting growth.

Extracts of western juniper and Alaska cedar heartwood both produced the greatest reductions in isolations at the middle concentration (0.05%), although the effect declined at the higher concentration. These oils appear to have some promise as topical surface mold treatments and merit further study. Both were also associated with effects on all of the genera isolated in the second trial, suggesting that they may have broader activity than other heartwood extracts. Interestingly, initial trials with nootkatone, a major component in Alaska cedar heartwood, produced little effect on fungal isolations. This suggests that other extractives in this species have a greater effect on molds and argues for using whole extracts with broader potential activity rather than individual components.

Foliage extracts also appeared to be effective, with Douglas-fir and western red-cedar leaf oil both producing inhibition of *Trichoderma* and some reductions in incidence of the other genera. Leaf extracts may be more attractive for these applications because they can be produced from the by-products of harvest or can be harvested without the need to cut trees. This might result in more sustainable resource utilization.

The results suggest that, while many woods are naturally resistant to microbial attack, extractives from these woods lack the broad spectrum activity needed to kill all of the fungi that can invade wood surfaces.

In some cases, the inability to reduce fungal isolations may reflect a tendency for the extracts to be fungistatic rather than fungicidal. Reisolation on fresh media, as was done in this test, removes the static effect, allowing fungi to grow. In addition, some isolations may reflect renewed fungal attack or regrowth from the fungus within the wood. Taylor *et al.* (2005) showed that bleach treatments could temporarily reduce surface molds, but incubation of treated material under non-drying conditions allowed for renewed colonization. Clearly any attempt to use natural products extracts as topical mold treatments must be coupled with drying to limit renewed attack.

CONCLUSIONS

The results suggest that a number of natural extracts can eliminate various fungi from wood surfaces; however, no single extract completely protected the wood. The use of multi-component extracts may represent a method for enhancing protection while creating the potential for reduced overall extract consumption.

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