Degree of discoloration by two *Ophiostoma* species in wounds in Douglas-fir trees

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Abstract

The rate of discoloration of Douglas-fir sapwood by two *Ophiostoma* species introduced through bark wounds was assessed in three separate trials over a 1-year period. Discoloration was most rapid in the longitudinal direction, reflecting the ability of the fungi to grow readily along the tracheids. Growth was more rapid in the radial than tangential direction, again reflecting the presence of more pathways for fungal ingress (i.e., the rays). The discoloration occupied 6 to 9 percent of the total section area 39 to 40 days after fungal inoculation illustrating the potential for fungal damage with prolonged log storage. Implications of this discoloration on log storage are discussed.

The bark on a living tree is an excellent barrier against invasion by an array of microorganisms, including many fungi. In addition to this physical barrier, the living tree is able to mount a host of physiological responses should this barrier fail. Once the tree is cut, however, the tree gradually loses the ability to respond to invasion and is eventually colonized by a diverse array of fungi, including many species that can permanently discolor the sapwood, reducing the aesthetic value of the resulting lumber. This process of invasion is facilitated by insects, notably beetles, such as the Scolytidae, that vector fungal spores between trees and logs; but, it can also be exacerbated by stem damage induced during harvesting, transport, or storage (Dowding 1984, Leach et al. 1934, Powell et al. 1995). The effect of such damage on the overall degree of discoloration is unclear. But it would seem that more heavily damaged trees would have more sapwood exposed to potential attack. There is, however, little information on the rates of discoloration around wounds on freshly felled trees, despite the potential for using this information to formulate strategies to reduce stain by scheduling logging activities to minimize log storage or incorporating post-harvest steps such as water sprinkling of log decks.

The relative rates of fungal discoloration by *Ophiostoma* spp. have been examined on: southern pine (Lindgren 1942), jack pine (*Pinus banksiana* Lamb.) (Yang and Beauregard 2000), lodgepole pine (*Pinus contorta* Dougl.) (Fleet et al. 1999), radiata pine (*Pinus radiata* D. Donn.) (Kreber et al. 1999, Uzunovic et al. 2004, O'Callahan et al. 2002, Butcher 1966, Drysdale et al. 1986, Keirle 1980), Corsican pine (*Pinus nigra* L.) (Lee and Gibbs 1996, Uzunovic et al. 1999a), and Scots pine (*Pinus sylvestris* L.) (Uzunovic et al. 1998, Uzunovic and Webber 1998, Jakobsson 1976). Growth rates of selected fungi have been studied in southern pines (Scheffer and Lindgren 1940), but there is little information on the rates of spread in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). Douglas-fir is a commercially important species in the Pacific Northwest, in part because of its attractive appearance. The presence of fungal discoloration can reduce value in some applications, particularly in a market that is increasingly sensitized to the presence of any fungi on wood-based materials. The presence of existing fungal colonization in logs means that any lumber cut from this material will be similarly colonized. While freshly sawn boards cut from heavily colonized logs may not have obvious fungal growth on the surface (as spores or hyphae), there is some evidence that limiting such growth by application of anti-stain chemicals is more difficult than for non-colonized materials (Scheffer and Lindgren 1940). This effect reflects the differences between controlling the growth of spores or hyphal fragments landing on wood surfaces and controlling growth of established hyphae from the interior to the wood surface.

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Developing better estimates of the rates of discoloration would allow forest operations personnel to assess the costs associated with more closely scheduled harvests and more careful handling of logs against the potential value of cleaner, less heavily colonized wood. In this paper, the relative rates of fungal discoloration of Douglas-fir trees by two *Ophiostoma* spp. are described.

Materials and methods

The tests were performed in the Spring, Summer, and Winter over a 1-year period on trees harvested near Corvallis, Oregon. The trees ranged from 100 to 250 mm in diameter at breast height (DBH) and were 10 to 15 m tall. All were grown on open sites, were 8 to 10 years old, and contained high percentages of sapwood. In all of the tests, the trees were harvested, delimbed, and cut into approximately 100- to 150-mm-long sections. The cross sections and branch stubs were coated with wax to retard drying and restrict fungal attack. All sections were inoculated within 10 to 12 hours of felling.

Once the sections were sealed, two 10-mm-diameter wounds were created through the bark and cambium in each section 180 degrees apart at the approximate midpoint leaving exposed xylem. One hundred µl of distilled water was added to one wound while the other received 100 µl of a suspension of the test fungus. *Ophiostoma piceae* (Munch) Syd & Syd and *Ophiostoma perfectum* (Davidson) deHoog were inoculated on 2 percent malt extract agar in petri dishes and incubated for 10 days in the dark at 25°C. These species were selected because they have been frequently isolated from freshly sawn lumber in the Western United States and Canada (Uzunovic et al. 1999b). The plates were flooded with sterile distilled water and the fungal spores and hyphae were dislodged by rubbing the agar surface. The resulting liquid was decanted and adjusted to a density of 10^6 spores and hyphal fragments per ml using a haemocytometer.

The sterile water and inoculated wounds were covered with duct tape after treatment to limit further fungal colonization, and this tape was covered with wax to retard moisture loss. The sections were then incubated in a laboratory at room temperature (21°C to 25°C) for varying time periods. At each selected time point, five or six sections inoculated with each fungus were removed from the test to assess the degree of fungal colonization.

In the first trial, a series of 5-mm-thick disks were cut along the length of each section so that the middle disk encompassed the original two wounds. The degree of visual fungal discoloration was measured radially, tangentially, and longitudinally to the nearest millimeter on each section. The discolored area in each section was calculated, then the areas from each disk were combined to produce an estimate of total volume of stained wood. The sections were then incubated for 24 hours at 20° to 23°C to allow the fungi colonizing the wood to grow out on the cross section. The radial, tangential, and longitudinal distances for this hyphal growth from the original point of inoculation were similarly measured. These measurements must be viewed with some caution since the fungus can grow onto the wood surface and then outward. This outward growth could produce larger estimates of fungal colonization.

The second trial used similar procedures but included an additional assessment of fungal attack made by cutting a series of the sections from the zones around the visibly discolored areas. These sections were then reacted with fluorescen isothiocyanate (FITC) conjugated wheat germ agglutinin, a lectin specific for the n-acetylglucosamine in chitin in the fungal hyphae (Morrell et al. 1985), mounted on glass slides, and examined using a light microscope equipped with a xenon lamp and filters specific for FITC. The distance that brown pigmented hyphae were detected and the relative percentage of cells colonized were estimated, then similar measurements were made on the fluorescing hyphae. Since pigmented hyphae do not fluoresce, we hoped to be able to distinguish younger, hyaline hyphae on the periphery of the damaged zone, from older more heavily pigmented hyphae closer to the wound.

The third test evaluated the ability of the test fungus to grow tangentially around the cambium, phloem, and first growth rings. Sections were prepared as described, but in this case, the degree of fungal colonization was assessed around the circumference both visually and microscopically. This experiment was initiated because preliminary observations suggested extensive cambial and phloem colonization which could be advantageous to the fungus both for excluding competing fungi and allowing the fungus access to nutrients in this zone.

Results and discussion

Non-fungal inoculated wounds tended to be free of discoloration, although there were a few exceptions. Discoloration of some controls probably represented surface contamination of the wounds during the short exposure period between wounding and sealing. Both fungi colonized the inoculated wounded sections relatively quickly (**Table 1**). As expected, the discoloration tended to form a wedge-shaped zone of discoloration, reflecting the tendency for the test fungi to preferentially grow

Table 1. — Relative radial, tangential, and longitudinal extent of discoloration and visible fungal growth of Ophiostoma piceae or Ophiostoma perfectum inoculated into wounds in Douglas-fir stem sections.

		Distance from inoculation point (mm)[a]								
		Radial			Tangential			Longitudinal		
Fungus	Fungal growth[b]	11 to 12 days	29 to 30 days	39 to 40 days	11 to 12 days	29 to 30 days	39 to 40 days	11 to 12 days	29 to 30 days	39 to 40 days
O. perfectum	Pigmented	7.0 (0.8)	7.8 (2.2)	20.0 (5.6)	2.8 (1.6)	2.8 (0.4)	12.6 (14.1)	12.6 (3.1)	16.7 (3.0)	26.6 (12.1)
	Total	9.2	15.2	24.2	9.0	12.0	19.2	18.1	29.0	40.5
O. piceae	Pigmented	6.8 (1.1)	17.6 (2.1)	19.0 (6.2)	4.2 (2.2)	7.6 (2.9)	13.8 (5.3)	18.8 (4.1)	26.0 (8.4)	46.0 (5.1)
	Total	11.0	21.4	24.8	9.6	16.4	17.4	26.9	26.0	60.0

[a] Values represent means of six measurements per time per fungus. Values in parentheses represent one standard deviation.
[b] Where pigmented represents distance that melanized hyphae were detected and total represents distance for both melanized and hyaline hyphae.

along the rays in the sapwood and longitudinally along the tracheids.

The zones of discoloration tended to increase steadily over the 30 to 40 days of testing. The degree of discoloration tended to be highest in the longitudinal direction, ranging from 21 to 65 mm for *O. perfectum* and 47 to 69 mm for *O. piceae* after 40 days. These rates reflect the ability of the fungus to grow more rapidly along the tracheids instead of tangentially through pits or the cell wall.

The degree of discoloration in the radial and tangential directions tended to be only 10 to 30 percent of that found longitudinally. Radial discoloration appeared to be greater than tangential, although the differences tended to diminish with time. Radial discoloration is limited by sapwood depth, while tangential discoloration can continue to extend around the circumference. As a result, the ratio of radial to tangential discoloration should decrease over time.

Effect of season of cutting on relative degree of discoloration

The results from tests of samples cut in the spring, summer, and winter showed that radial discoloration was slightly greater in the logs cut in the summer than in the other two seasons (**Table 2**). Spring harvested samples experienced much greater discoloration when exposed to *O. perfectum* for 18 days. The reasons for this large increase in discoloration in spring harvested materials is unclear, although it may reflect seasonal variations in stored carbon compounds as the trees initiate new needle growth.

Discoloration vs. fungal colonization

Hyphal melanization provides the most visible evidence of fungal attack; however, this process only occurs as hyphae mature (Zink and Fengel 1988, 1989, 1990; Zabel and Morrell 1992). Fungal growth should tend to extend some distance beyond this area of obvious stain and can serve as an indication of future discoloration. With three exceptions, hyphal growth tended to exceed discoloration in all directions at all times, but the differences were sometimes slight (**Table 1**).The relatively small differences observed imply that melanization occurs rapidly in these two fungal species.

Percentage of wood discolored

While measuring the absolute distances that each fungus grew in a given direction is useful, a more practical measure is the total volume of wood that is discolored. The estimated volumes of wood that were discolored 11 or 12 days after wounding and inoculation were fairly small, ranging from 1 to 4 percent (**Fig. 1**). Average discolored areas for *O. piceae* and *O. perfectum* were 1.96 and 2.89 percent, respectively. Discolored areas increased for both fungi 29 to 30 days after wounding (means 5.29% and 3.11% for *O. piceae* and *O. perfectum*, respectively), but the differences in discolored areas among individual samples increased markedly. The increased variation reflects differences in the wood samples in terms of nutrient levels and moisture contents as well as relative differences in fungal growth. Mean discolored areas after 39 to 40 days increased to 9.11 and 6.07 percent of the section area for *O. piceae* and *O. perfectum*, respectively. Individual values varied more widely, ranging from 1 to 16 percent.

While the overall discoloration percentage of the sections remained small, (< 10%) it must be remembered that the discoloration originated from one small wound. Repeated rough handling of logs during felling, skidding, yarding, and transport creates ample opportunity for multiple wounding. In addition, the test period was relatively short in comparison with typical processing times for logs of this species. Logs in the Pacific Northwest may be stored in decks for periods ranging from one to several months, during which time stain fungi can continue to discolor wood. Previous studies have also noted substantial effects of mechanized harvesting in terms of wound creation as well as serving as a vector for fungal inoculum (Uzunovic et al. 2004). Clearly, prolonged storage can result in considerable fungal attack under the proper conditions.

Table 2. — Directional growth of Ophiostoma piceae and Ophiostoma perfectum 18 days after inoculation into wounds in freshly cut Douglas-fir harvested at three different times of the year.

	Fungal growth (mm)			
	Radial		Tangential	
Harvest season	*O. piceae*	*O. perfectum*	*O. piceae*	*O. perfectum*
Spring 2003	3.8	5.0	3.5	14.2
Summer 2003	6.8	7.0	4.2	2.8
Winter 2003–2004	3.8	2.8	2.0	0.9

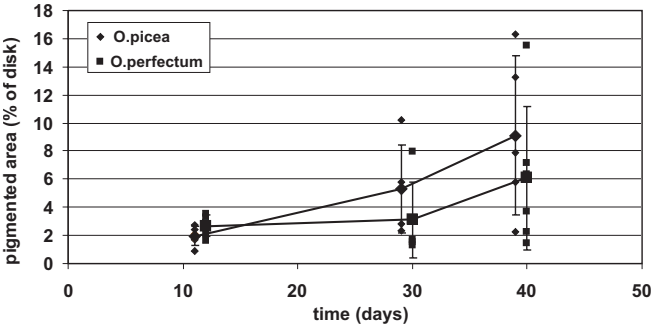

Figure 1. — Percentage of discolored wood area around wounds inoculated with Ophiostoma piceae and Ophiostoma perfectum 11 to 40 days earlier. Error bars represent one standard deviation while individual points represent outliers.

Cambial colonization by *O. piceae* or *O. perfectum*

Observations of sections from initial trials suggested that the *Ophiostoma* spp. initially preferentially colonized the very outer sapwood ring, phloem, and cambium before growing further into the wood (**Fig. 2**). This growth pattern might allow the fungus to sequester nitrogen and readily assimilate carbon stored in these zones. It might also limit colonization by competing fungi. In order to measure this growth, additional test sections were established, but observations were confined to the presence of hyphae in the tangential direction away from the point of inoculation. Tangential growth of non-pigmented hyphae around the cambium was extremely limited for both fungi. Tangential growth increased to 2 mm over the first 18 days for *O. piceae* and to 1 mm for *O. perfectum*. The limited growth of hyaline hyphae in these tests suggests that these stain fungi did not preferentially colonize the cambium but instead grew more readily in the radial and longitudinal directions. As a result, the size of the wound remains the primary factor in de-

Figure 2. — Cross section of Douglas-fir 40 days after inoculation of a wound with Ophiostoma piceae showing complete discoloration of the first growth ring and a wedge-shaped discoloration pattern further in from the wounded area at the bottom of the section.

termining the degree of fungal colonization at the earlier stages of log storage. This effect would diminish over time as longitudinal growth from numerous wounds result in coalescing of discolored areas.

Implications

Fungal colonization of freshly felled trees has long been an issue for producers attempting to limit discoloration, but the effects of handling damage on this process has received little attention. Both *O. piceae* and *O. perfectum* were capable of discoloring substantial areas around Douglas-fir wounds. This damage can reduce value and make it more difficult to limit subsequent fungal growth. Solutions to these problems include more timely processing and attempts to limit bark damage in materials in the forest. Other possible approaches include application of fungicides or biological control agents to limit colonization or the use of either sprinkling or ponding to limit attack during storage. Both of these approaches can be difficult to employ in many locations. Chemical application to logs in the woods is difficult and probably not acceptable to regulatory authorities or many companies. Water treatments are relatively simple, but can entail regulatory issues with surface discharge of the water. Given these constraints, timely processing remains the primary tool for minimizing log discoloration.

Conclusions

Fungal discoloration around wounds in Douglas-fir stems caused by two *Ophiostoma* species covered up to 10 percent of the area within 40 days after wounding. While there were differences in rates of discoloration between the two fungi tested and between samples harvested at different times of the year, the overall rates of discoloration highlight the importance of timely processing to minimize discoloration in stored logs. It is important to note that these trials were performed on relatively small-diameter trees; results in larger trees might differ due to differing physiological response capabilities to fungal invasion.

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