Pathogenicity to Douglas-fir of *Ophiostoma pseudotsugae* and *Leptographium abietinum*, fungi associated with the Douglas-fir beetle

Darrell W. Ross and Halvor Solheim

Abstract: Pole-size Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) were inoculated with two fungi (*Ophiostoma pseudotsugae* (Rumb.) von Arx and *Leptographium abietinum* (Peck) Wingf.) associated with the Douglas-fir beetle (*Dendroctonus pseudotsugae* Hopkins) to evaluate their pathogenicity. Pruning the lowermost 30% of the live crown had no effect on host tree defenses. Inoculation with *O. pseudotsugae* produced significantly longer lesions in the phloem and resulted in a significantly greater percentage of necrotic phloem than inoculation with *L. abietinum*. The percentage of occluded sapwood was also greater following *O. pseudotsugae* inoculations, but the difference was not statistically significant. Individual lesion lengths declined significantly with increasing inoculation density, but the total percentage of necrotic phloem increased significantly. Both fungi appeared to be better adapted to grow in sapwood than in phloem. None of the inoculated trees were dead after 5 months, but some were chlorotic with less than 30% functional sapwood within the inoculation band. The results suggest that these fungi may assist the Douglas-fir beetle in overcoming the defenses of live trees.

Résumé : Des Douglas taxifoliés (*Pseudotsuga menziesii* (Mirb.) Franco) au stade de perchis ont été inoculés avec deux champignons, *Ophiostoma pseudotsugae* (Rumb.) von Arx et *Leptographium abietinum* (Peck) Wingf., associés au dendroctone du Douglas taxifolié (*Dendroctonus pseudotsugae* Hopkins) dans le but d'évaluer leur pathogénécité. Le fait d'élaguer 30% de la cime dans sa partie inférieure n'avait pas d'effet sur les réactions de défense de l'hôte. L'inoculation avec *O. pseudotsugae* a causé des lésions significativement plus longues dans le phloème et un pourcentage significativement plus élevé de phloème nécrotique qu'avec *L. abietinum*. Le pourcentage de bois d'aubier obstrué était également plus élevé dans le cas des inoculations avec *O. pseudotsugae* mais la différence n'était pas statistiquement significative. La longueur des lésions diminuait significativement avec l'augmentation de la densité des inoculations mais le pourcentage total de phloème nécrotique augmentait de façon significative. Les deux champignons semblaient mieux adaptés pour se développer dans le bois d'aubier que dans le phloème. Aucun des arbres inoculés n'était mort après 5 mois mais certains étaient chlorosés et avaient moins de 30% de bois d'aubier fonctionnel dans la zone d'inoculation. Les résultats suggèrent que ces champignons pourraient aider le dendroctone du Douglas taxifolié à surmonter les défenses des arbres vivants.
[Traduit par la Rédaction]

Introduction

Most bark beetles vector one or more species of blue-stain fungi (Whitney 1982; Harrington 1988). The relationships among the beetles, fungi, and host trees are highly variable (Harrington 1993*a*, 1993*b*). Some of these fungi are clearly pathogenic and may assist the beetles in overcoming host tree defenses (Nelson and Beal 1929; Horntvedt et al. 1983; Christiansen 1985*a*; Owen et al. 1987; Solheim 1988; Nebeker et al. 1993). However, some beetles lack fungal associates (Lieutier 1993) and others are apparently capable of killing host trees in the absence of their normal associates (Bridges et al. 1985). It has been suggested that the fungi, in some cases, may reduce the quality and quantity of potential habitat for the beetle (Barras 1970; Franklin 1970; Bridges and Perry 1985).

Two species of blue-stain fungi commonly associated with the Douglas-fir beetle (*Dendroctonus pseudotsugae* Hopkins) are *Ophiostoma pseudotsugae* (Rumb.) von Arx and *Leptographium abietinum* (Peck) Wingf. (Rumbold 1936; Harrington 1988; Lewinsohn et al. 1994; H Solheim, unpublished). These fungi are transported within pits on the surface of the exoskeleton of adult beetles of both sexes (Lewinsohn et al. 1994). There is only one published report on the pathogenicity of either fungus to the host tree. Two-year-old Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings inoculated with *L. abietinum* (=*Verticicladiella abietina* (Hughes) Kendr.) isolated from grand fir (*Abies grandis* (Dougl.) Lindl.) showed no signs of disease and remained alive (Harrington and Cobb 1983). The objective of our study was to assess the pathogenicity of *O. pseudotsugae* and *L. abietinum* to Douglas-fir.

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D.W. Ross. Department of Forest Science, Oregon State University, Corvallis, OR 97331, U.S.A.
H. Solheim. Norwegian Forest Research Institute, Section of Forest Ecology, Hogskolevn, 12, N-1432 Ås, Norway.

Materials and methods

This study was conducted in a 14-year-old Douglas- fir plantation on the Siuslaw National Forest near Florence, Oregon (approximately 44°N, 124°W). The site is about 2.5 km from the Pacific Ocean, 122 m above sea level, and representative of the *Picea sitchensis* vegetation zone (Franklin and Dyrness 1973).

On May 23, 1993, 48 trees were selected to be as uniform as possible based on general appearance and size. From a random starting point, the lowermost whorls of branches were pruned from alternate trees removing approximately 30% of the live crown (Table 1). The pruning treatment was applied to produce trees with lower

Table 1. Characteristics of Douglas-fir trees inoculated with *Ophiostoma pseudotsugae* or *Leptographium abietinum* on the Siuslaw National Forest, Oregon.

Pruning category	DBH (cm)	Tree height (m)	Live crown length (m)	No. of live branch whorls
Unpruned	14.1±0.3	11.1±0.2	7.4±0.2	8.2±0.1
Pruned	12.6±0.5	10.3±0.3	5.2±0.2	5.8±0.1

Note: Values are means of 24 observations ± SE.

resistance to fungal invasion than the unpruned trees (Christiansen and Fjone 1993; Långström et al. 1993).

Each pruned and unpruned tree was randomly assigned to one of six inoculation treatments. The treatments were *O. pseudotsugae* or *L. abietinum* at 200, 400, or 800 inoculation sites/m^2 of bark surface. Fungi used for the inoculations were isolated in British Columbia in August 1992 from trees infested with the Douglas-fir beetle. Trees were inoculated on June 8–9, 1993, in a 60 cm tall band around the circumference of the tree at a height between 1 and 2 m. The specific height of the inoculation band varied slightly to avoid branch whorls and minor bole defects. Trees were inoculated by the cork-borer technique using a 5 mm diameter borer (Wright 1933; Christiansen 1985*b*). Inocula were 20-mm^3 plugs of malt agar (2% malt, 1.5% agar) containing mycelia from 2-week-old cultures. No sterile wound controls were included because studies with a variety of blue-stain fungi and conifer hosts have repeatedly shown that they produce only small lesions (Molnar 1965; Reid et al. 1967; Horntvedt et al. 1983; Raffa and Smalley 1988; Solheim 1988; Parmeter et al. 1989).

The trees were felled on November 4–5, 1993. Before felling, the outer bark was removed over three of the uppermost and three of the lowermost inoculation sites, exposing the lesion in the phloem. The total length of the lesion in the phloem just beneath the outer bark was measured. In some cases, the lesions had coalesced (7.3% of those measured), so they were measured only in the direction away from the inoculation band. These measurements were doubled to estimate the total lesion length. After felling, the central 30-cm section of the 60 cm wide inoculation band was removed and taken to the laboratory.

Thin cross sections (about 5 mm) of the bole segments were cut 10 cm from the center in both directions. The areas of healthy, functioning sapwood (wet) and partially or entirely occluded sapwood (dry) were traced immediately on each cross section based upon translucency (Solheim et al. 1993). Each cross section was photocopied along with a ruler, the photocopies were digitized on an optical scanner, and the areas of healthy and occluded sapwood were determined with image analysis software (SigmaScan/Image, Jandel Scientific, San Rafael, Calif.). The outer bark was removed from a 15 × 15 cm area of the central 20-cm bole section and all lesions were traced onto clear acetate. The total lesion area was determined from the acetate sheets following the same procedure used to determine the areas of healthy and occluded sapwood.

At three inoculation sites on each stem section, small pieces of sapwood (5–15 mm^3) were removed aseptically from just beneath the bark, at the midpoint of the occluded sapwood, and at the inner margin of the occluded sapwood. Each piece of sapwood was placed on a plate of malt agar and incubated in darkness at room temperature. The plates were monitored for 6 weeks for the presence of the inoculated fungi.

The mean lesion length, percent necrotic phloem, and percent occluded sapwood were subjected to analysis of variance (ANOVA) for a factorial design with pruning, fungal species, and inoculation density as factors (Steel and Torrie 1980). Before ANOVA, each variable was transformed to ln(Y) to correct for heteroscedasticity in the untransformed data as indicated by graphical analysis of residuals (Neter et al. 1983). The nontransformed means are reported. Means were compared and separated by Fisher's protected least significant difference test (Steel and Torrie 1980). All statistical analyses were performed with SAS computer programs (SAS Institute Inc. 1985).

Table 2. Percent positive reisolations of *Ophiostoma pseudotsugae* and *Leptographium abietinum* from Douglas-fir sapwood following artificial inoculations.

Sample location	*O. pseudotsugae*	*L. abietinum*
Outermost sapwood	66.7	93.1
Midway through occluded sapwood	23.6	48.6
Inner margin of occluded sapwood	11.1	23.6
Total	72.2	100.0

Note: Each value is based on 72 samples. Total is the percentage of inoculation sites with at least one positive reisolation for the three individual samples.

Results and discussion

Both fungi were reisolated from a high percentage of the samples taken from the outermost sapwood directly beneath the inoculation sites (Table 2). The reisolations were less successful from samples taken midway through or at the inner margin of the occluded sapwood. For all sample locations, *L. abietinum* was reisolated more frequently than *O. pseudotsugae*. One sample from the inner margin of occluded sapwood in a tree inoculated with *L. abietinum* also contained *O. pseudotsugae*. This was most likely due to contamination in the laboratory during reisolation. These results indicate that most of the inoculations were successful with apparently no contamination.

None of the interactions among factors were significant for any variables, nor did pruning have a significant effect on any of the variables ($P > 0.05$). In similar studies with Scots pine (*Pinus sylvestris* L.) in Norway and Sweden, removing the lower 30% of the live crown had no effect on tree resistance, but removing 50–75% of the live crown significantly reduced tree resistance (Christiansen and Fjone 1993; Solheim et al. 1993). We originally intended to remove at least 50% of the live crown, but because of limitations of our equipment and the size of the trees, we were able to remove only 30%. Considering the favorable conditions for tree growth at the study site (i.e., moderate temperatures, abundant moisture, and fertile soil), it is not surprising that the relatively light pruning had no apparent effect on tree resistance.

Inoculation with *O. pseudotsugae* resulted in significantly longer lesions and greater percent necrotic phloem than inoculation with *L. abietinum* (Table 3). Percent occluded sapwood was also greater following inoculation with *O. pseudotsugae* than inoculation with *L. abietinum*, but the difference was not statistically significant (Table 3). The nonsignificant effect of fungal species on percent occluded sapwood may have been due to the small number of replications or may indicate differences between the fungi in their ability to grow in phloem and xylem tissues. For example, among the blue-stain fungi associated with *Ips typographus*, *Ophiostoma penicillatum* grows most rapidly in the phloem, but *Ophiostoma polonicum* grows most rapidly in the xylem (Solheim 1988).

The length of individual lesions declined significantly with increasing inoculation density (Table 3). The smaller lesion size at higher inoculation densities probably reflects the

Fig. 1. Cross section of a Douglas-fir bole inoculated with *Ophiostoma pseudotsugae* at 400 sites/m^2 illustrating the irregular pattern of sapwood occlusion. Blackened portions of the sapwood were partially or totally occluded.

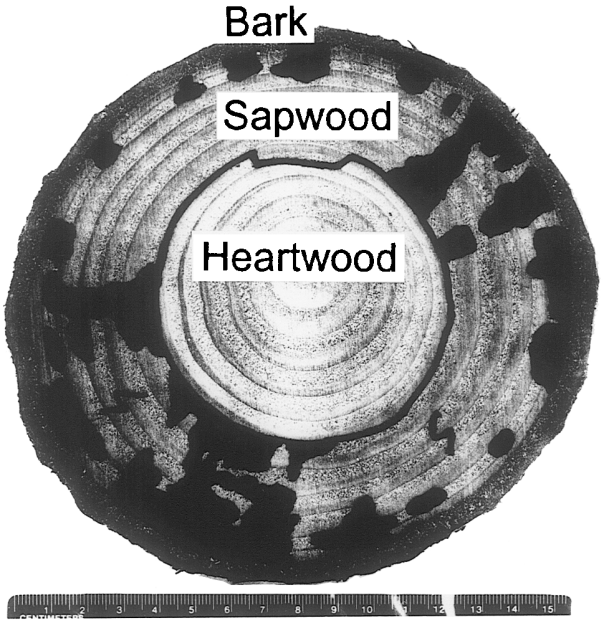


Table 3. Mean lesion length, percent necrotic phloem, and percent occluded sapwood in Douglas-fir following artificial inoculations of *Ophiostoma pseudotsugae* or *Leptographium abietinum*.

Factor	Lesion length (mm)	% necrotic phloem	% occluded sapwood
Fungal species			
O. pseudotsugae	59.9*a*	15.9*a*	38.7*a*
L. abietinum	23.1*b*	5.1*b*	30.2*a*
P-value	0.0001	0.0001	0.1385
Inoculation density			
200/m^2	46.1*a*	6.5*a*	28.3*a*
400/m^2	41.3*ab*	10.3*b*	33.7*a*
800/m^2	37.1*b*	14.6*b*	41.3*a*
P-value	0.0433	0.0014	0.1402

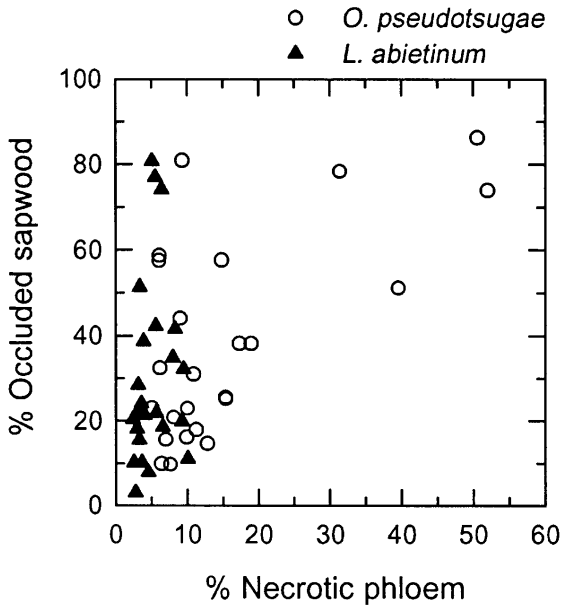
Note: For fungal species and inoculation density, values followed by the same letter are not significantly different by Fisher's protected LSD ($P > 0.05$).

limited defensive capacity of the host trees. Although small lesions usually indicate a rapid and effective defensive response, they can also arise from a depletion of resources available for defense (Christiansen and Horntvedt 1983; Raffa and Berryman 1983; Christiansen 1985*b*; Christiansen et al. 1987).

Although individual lesion size declined with increasing inoculation density, the total amount of necrotic phloem increased significantly (Table 3). Apparently, the smaller size of individual lesions was compensated for by the higher inoculation densities. Percent occluded sapwood also increased with increasing inoculation density, but the differences were not statistically significant (Table 3).

In previous studies with Douglas-fir, *O. polonicum* grew tangentially in the sapwood to a greater extent than in other

Fig. 2. Relationship between percent occluded sapwood and percent necrotic phloem in Douglas-fir inoculated with either *Ophiostoma pseudotsugae* or *Leptographium abietinum* ($n = 48$).



host tree species (Christiansen and Solheim 1990, 1994). The fungus was restricted to small necrotic lesions in the phloem, but affected more than 45% of the sapwood in three of the four inoculated trees (Christiansen and Solheim 1990). We observed a similar pattern following inoculations of Douglas-fir with *O. pseudotsugae* and *L. abietinum*. In contrast with the wedge-shaped patterns often observed following inoculation of conifer hosts with blue-stain fungi, the pattern in Douglas-fir was irregular, resulting from various degrees of tangential growth (Fig. 1). Although a few trees produced large lesions in the phloem, the majority were rather small in comparison with those produced in other tree species. In some trees with small lesions, a high percentage of the sapwood was occluded (Fig. 2).

The inoculation densities used in this study were higher than typical Douglas-fir beetle attack densities (<100/m^2) (McMullen and Atkins 1961; Hedden and Pitman 1978). However, the inoculations were applied only to a 60 cm long section of the bole, which is much less than would be occupied by attacking beetles (Furniss 1962). Consequently, the total number of inoculations applied to each tree was far below the number of attacks that would occur during a beetle infestation. Previous studies have indicated that the total number of inoculations per tree may be more important than the inoculation pattern (Christiansen 1985*a*; Horntvedt and Solheim 1991).

Our results suggest that these fungi may help Douglas-fir beetles to overcome the resistance of live trees. Although none of the trees were dead at the time of harvest, the appearance of several trees indicated that their death was imminent. It is likely that some of these trees would have died, if we had delayed harvesting. For example, one of the smaller trees (DBH = 9.6 cm) that received the high-density inoculation of *O. pseudotsugae* was obviously chlorotic and only 14% of the sapwood remained functional at the time of harvest. Additionally, our results suggest that *O. pseudotsugae* may be more

aggressive in Douglas-fir than *L. abietinum*. Further studies will be necessary to determine whether these fungi are essential for the Douglas-fir beetle to successfully breed in live trees.

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