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Pathogenicity to Western Larch (Larix occidentalis) of Two Fungi,
Ophiostoma pseudotsugae and Leptographium abietinum, Associated with the
Douglas-fir Beetle (Coleoptera: Scolytidae) in Eastern Oregon, U.S.A.

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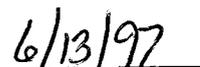
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An Undergraduate Thesis in Bioresource Research

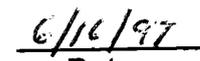
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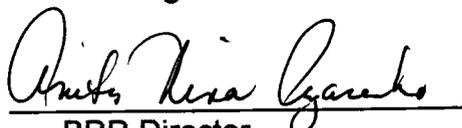
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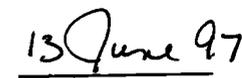

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Abstract: Pole-sized, live western larch (Larix occidentalis Nutt.) were inoculated with Ophiostoma pseudotsugae (Rumb.) von Arx or Leptographium abietinum (Peck) Wingf., two blue stain fungi associated with the Douglas-fir beetle (Dendroctonus pseudotsugae Hopkins), to assess their pathogenicity. Inoculation with O. pseudotsugae resulted in a significantly greater percentage of necrotic phloem compared to inoculations with L. abietinum. The percentage of occluded sapwood was significantly greater after L. abietinum inoculations compared to O. pseudotsugae inoculations. Percentages of necrotic phloem and occluded sapwood were positively correlated with inoculation density. In the inoculation band, all trees had greater than 60% functional sapwood four months after inoculation. The results suggest that western larch can successfully limit colonization by O. pseudotsugae and L. abietinum. The inability of the fungi to thrive in live western larch may be a factor in the consistent failure of Douglas-fir beetle brood to survive in live western larch.

Introduction

The Douglas-fir beetle, Dendroctonus pseudotsugae Hopkins, at high densities, can cause significant mortality of Douglas-fir, Pseudotsugae menziesii (Mirb.) Franco, adversely affecting forest resource values (Cornelius 1955; Rudinsky 1966; Johnson and Belluschi 1969; Furniss and Orr 1978). For example, in 1978, Douglas-fir beetle outbreaks caused the loss of more than one million m³ of lumber in the western United States (Furniss and Orr 1978; Brown and Orr 1980). In addition to the obvious

impact on the timber resource, changes in stand structure and composition resulting from beetle infestations may also affect wildlife, recreation, and other natural resource values.

Tree death due to bark beetle attack is different from tree death due to defoliation, debarking, felling, or other causes. Bark beetle-caused mortality begins when beetles enter the phloem, inoculating fungi that cause tissue death near the entrance holes. This is followed by termination of translocation, thus the crown of the tree soon becomes water stressed, vital functions dwindle, and the tree rapidly dies (Whitney 1982). Tree death due to bark beetle attack has instigated the question: Who is primarily responsible the beetle or the fungus that it carries? Some research suggests the pathogenicity of the many species of blue-stain fungi carried by numerous bark beetle species is sufficient for tree death (Solheim 1988, Hortvedt and Solheim 1991, Solheim 1993). Other research suggests blue-stain fungi may not be required for bark beetle success (Barras 1970).

Similar to many macroorganisms, bark beetles exist with many different microorganisms inside and on the surface of their bodies. About 100 species of microorganisms have been found to be associated with bark beetles, but only a few of the 38 genera of fungal associates are understood as important symbionts (Whitney 1982). The blue-stain fungi commonly associated with most bark beetle species can grow as spores and as hyphae or mycelium. The spores become available as food for some beetle species. The hyphae or mycelium invades host sapwood and phloem adjacent to beetle tunnels, thus killing tree tissues beyond the immediate vicinity of the beetles.

In space and time, the relations between the bark beetle and its associated microorganisms are more than casual. In a broad sense symbiosis is defined by deBary (1879) as the living together of two or more organisms of different species. At

least three types of symbiosis are possible with regards to bark beetles and their associated fungi: mutualism, when both organisms benefit from the partnership, antagonism, when one or both organisms sustain damage, and commensalism where one organism benefits and the other is unaffected. Possible factors associated with antagonism include inhibition of bark beetle mating and oviposition, and poor brood development most likely due to decreases in the nutritional quality and/or quantity of the phloem available (Barras 1970). Factors associated with mutualism in bark beetles include vectoring of host tree pathogens, enhancing beetle nutrition, production of beetle pheromones, overcoming host tree defenses, and finally, the killing of host tree tissues. The primary benefit the fungi receives from the beetle is facilitation in dispersal, inoculation, and penetration into fresh substrate.

A possible benefit some beetle species receive from the fungi is enhancement of the nutritional value of the host phloem. Barras (1973) illustrated that when the southern pine beetle, Dendroctonus frontalis Zimm., is carrying its associated fungi, the beetles produce significantly more progeny in less time compared to beetles that are not carrying the fungi. The sequence of events of a bark beetle attack is such that the adults will have completed their reproductive activity before the fungi extensively colonize host tree tissues; hence, this function of mutualism is more beneficial to the larval stage or the maturation stage of young adults. This beneficial relation is dependent on the inherent nutritional status of the host phloem. This could result in widening the range of host tree nutritional conditions the beetle will exploit.

A second possible role for the associated fungi involves behavioral chemicals. Upon landing on a suitable host tree, most bark beetles release aggregation pheromones that stimulate a mass attack by conspecifics. Some fungi alter host tree compounds, producing substances that alter or enhance the action of aggregation or antiaggregation pheromones (Brand et al. 1976, Brand and Barras 1977).

Third, a major aspect of this symbiosis is the ability of the fungi and beetle to overcome host tree defenses. Bark beetle success in live trees depends on their ability to overcome host defenses (Berryman 1972; Raffa and Berryman 1983). Conifer defense systems have two known components: preformed resin flowing from severed resin ducts and an induced wound response (Reid et al. 1967; Berryman 1972). The induced wound response is a hypersensitive reaction in the tissue surrounding the wound. It restricts the invading insect or pathogen by accumulating toxic or inhibitory compounds such as terpenes (Berryman 1972). This reaction is characterized by rapid cellular desiccation and necrosis around the wound site and the synthesis and release of terpenes and other compounds by the dying parenchyma cells (Miller 1950; Reid et al. 1967; Berryman 1969). The dimensions of the induced wound response of the host generally correlate to the extent of fungal growth, as well as to the damage done by tunneling of the beetles (Ross et al. 1992; Lieutier 1993; Solheim 1993). The joint action by the bark beetle and its associated fungi can deplete host tree defensive resources resulting in successful colonization and tree death.

The fourth function of this symbiosis is tree death. Living tree tissues prevent successful beetle reproduction as described above, but changes in gas exchange and tree moisture that occur as the tree dies are extremely beneficial to the brood of the beetle and to the growth of the fungi (Webb and Franklin 1978). The host tree energy reserves, overall health of the tree, quality and quantity of the blue-stain fungi inoculated into the tree, as well as the density and distribution of beetle attack sites are all important factors in tree death.

Most bark beetles are secondary pests, attacking only physiologically weakened or dead trees (Kozlowski 1969). When populations reach high densities, however, many bark beetle species, including the Douglas-fir beetle, can attack and kill healthy, vigorous trees. Phloem of physiologically weak or downed Douglas-fir is the preferred

breeding site for this species. Occasionally, the beetle will also attack western larch, Larix occidentalis Nutt. (McCowan and Rudinsky 1958; Furniss 1962; Furniss et al. 1981). Although brood development in downed western larch is similar to that in Douglas-fir, successful brood development has not been observed in live western larch (Ross 1967; Furniss 1962).

The two species of blue-stain fungi most commonly associated with the Douglas-fir beetle are Ophiostoma pseudotsugae (Rumb.) von Arx and Leptographium abietinum (Peck) Wingf (Rumbold 1936; Harrington 1988). The pathogenicity of these fungi to host trees and their ecological relations with the beetle remain unknown. If these fungi play a role in overcoming the host tree response to Douglas-fir beetle attacks, then the inability of the fungi to thrive in live western larch may be a factor in the failure of brood development.

The goal of this study was to evaluate the effect of host-tree defenses on colonization of O. pseudotsugae and L. abietinum. Measurements of induced wound response of western larch in response to artificial inoculations with these fungi were used to assess the intensity of host-tree defensive responses. These measurements, along with extent of sapwood occlusion, were used to evaluate the extent of fungal growth.

Materials and Methods

This study was conducted in a mixed conifer stand 16 km east of La Grande, Oregon, U.S.A., on the Wallowa-Whitman National Forest (45° N, 118° W). The elevation was about 1275 m. The stand consisted primarily of western larch with scattered Douglas-fir, ponderosa pine (Pinus ponderosa Dougl.exLaws), and grand fir (Abies grandis (Dougl.) Lindl.). Forty-eight trees were selected based on uniformity in general appearance and size. The mean (\pm standard error) diameter at breast height

was 13.4 ± 1.3 cm, height was 16.4 ± 1.4 m, and age was 63.3 ± 5.0 years. Each tree was randomly assigned to one of six treatments. The treatments were inoculation with *O. pseudotsugae* or *L. abietinum* at 200, 400, or 800 sites per m² of bark surface. The experimental design was a two by three factorial.

The trees were inoculated on 6 and 7 July 1995 with 2-week-old mycelium grown on 2% malt extract agar. The fungi were isolated in August 1992 from Douglas-fir-beetle-infested trees in British Columbia and maintained at the Norwegian Forest Research Institute, Ås, Norway. Trees were inoculated in a 60-cm-wide band between heights of 1 and 2 meters on the bole. Slight variation in height of the inoculation band was to avoid minor bole defects. The cork-borer method was used to inoculate the trees (Wright 1933; Christiansen 1985).

On 4 and 5 November 1995, the outer bark was shaved from three of the highest and three of the lowest inoculation sites on each tree to expose the induced lesions at the outer surface of the phloem. Lesion lengths were measured. The trees were felled, and the central 40-cm-long section of the inoculation band was removed for dissection. The bolts were stored at 0°C until processed.

Two five-mm-thick cross-sections were removed five cm in from both ends of each bole segment. Areas of functioning sapwood and partially or completely occluded sapwood were immediately traced on the cross-sections based on translucency (Solheim et al. 1993). Each cross-section was photocopied and digitized on an optical scanner. Areas of healthy and occluded sapwood were determined with image analysis software (SigmaScan / Image, Jandel Scientific, San Rafael, California). The outer bark was removed from a 225-cm² area of the remaining central 30-cm bole section and all exposed lesions at the outer phloem surface were traced onto clear acetate. The total lesion area was determined from the acetate sheets following the same procedure used to analyze the sapwood cross-sections.

Two small pieces of sapwood (5-15 mm³) were aseptically removed at three inoculation sites on each bole section. One piece was removed from just beneath the bark and the other from the inner margin of the occluded sapwood. Each piece was placed on 2% malt extract agar and incubated in the dark at room temperature. The plates were visually inspected for five weeks for presence of the inoculated fungi.

The mean lesion lengths, percentage of necrotic phloem, and percentage of occluded sapwood were analyzed by analysis of variance (ANOVA) for a factorial design, with fungal species and inoculation density as factors. The data were log transformed before ANOVA to correct for heteroscedasticity. Back-transformed means are reported. All statistical analyses were performed with SAS computer programs (SAS Institute Inc., Cary, NC 1994).

Results

Reisolations were highly successful for both fungi, ranging from 84 to 99% positive reisolations (table 1). For O. pseudotsugae, sampling from just beneath the inoculation site was slightly more successful than sampling from the inner margin of the occluded sapwood. For both sample locations, L. abietinum was isolated more frequently than was O. pseudotsugae.

No significant interactions were found between fungal species and inoculation densities for any of the variables ($P > 0.56$). Lesion lengths at the outer phloem surface were significantly greater for O. pseudotsugae inoculations than for L. abietinum ($P = 0.0001$, table 2). Inoculation with O. pseudotsugae resulted in a significantly greater percentage of necrotic phloem than did inoculation with L. abietinum ($P = 0.0001$, table 2). In contrast, inoculation with L. abietinum resulted in a significantly greater percentage of occluded sapwood than did inoculation with O. pseudotsugae ($P = 0.0002$, table 2). Reported occluded sapwood percentages are averages of upper and lower

sapwood measurements. There were no significant differences between the upper and lower occluded sapwood percentages ($P >$), so reported values are overall averages.

No significant correlation was found between inoculation densities and lesion lengths ($P=0.3437$, table 2). Both the percentage of necrotic phloem and the percentage of occluded sapwood increased significantly with increasing inoculation density (for necrotic phloem $P=0.0001$, for occluded sapwood $P=0.0102$, table 2).

Discussion

Densities of Douglas-fir beetle attacks are typically less than the inoculation densities used in this study (often $<100/m^2$) (McMullen and Atkins 1961). Nonetheless, the inoculations were only applied to a 60-cm-wide portion of the bole, which is a much smaller area of the tree than attacking Douglas-fir beetles would occupy (Furniss 1962). Thus, the total number of inoculation sites on each tree was well below the total number of attacks that would occur during an infestation.

Inoculation with *O. pseudotsugae* resulted in both significantly longer lesions and a higher percentage of necrotic phloem than did inoculation with *L. abietinum* (table 2). In contrast, the percentage of occluded sapwood was significantly lower after inoculation with *O. pseudotsugae* compared to inoculation with *L. abietinum* (table 2). These differences suggest that the two fungal species differ in their ability to grow in the phloem and xylem tissues of western larch. Similar differences in ability to invade host tissues have been observed among the blue-stain fungi associated with *Ips typographus*. In that case, *Ophiostoma penicillatum* grows most rapidly in the phloem, while *O. polonicum* grows most rapidly in the xylem (Solheim 1988). In our case, *Leptographium abietinum* appears to be better adapted to grow in the moist tissues of the xylem.

No significant correlation was found between lesion lengths and inoculation densities in western larch in our study ($P > 0.3437$, table 2). In contrast, Ross and Solheim (1997) reported the length of individual lesions declined with increasing inoculation densities for the same fungal species in Douglas-fir. The conflicting results may reflect differences in the defensive capacities of western larch and Douglas-fir relative to the fungal doses applied. Small lesions usually indicate a rapid and effective defensive response, with the fungi contained in the lesions. However, small lesions may also arise from a depletion of resources available for defense, with the fungi likely present beyond the margins of the lesions (Christiansen and Horntvedt 1983; Raffa and Berryman 1983). Regardless, lesion length can be misleading because it does not account for the intensity or the rate of the defensive reaction or the location of the fungi in relation to the lesion (Ross et al. 1992; Dunn and Lorio 1992).

The mean percentages of occluded sapwood reported in the Douglas-fir inoculation study by Ross and Solheim (1997) ranged from 28.3 to 41.3% for inoculation densities of 200, 400, and 800 sites per m². In our study, the mean percentages of occluded sapwood observed for western larch ranged from 3.7 to 7.6% for inoculation densities of 200, 400, and 800 sites per m² (table 1). The mean percentages of necrotic phloem were similar in the Douglas-fir inoculation study (15.9% following inoculation with *O. psuedotsugae* and 5.1% following inoculation with *L. abietinum*) and our western larch inoculation study (14.6% following inoculation with *O. psuedotsugae* and 6.0% following inoculation with *L. abietinum*). The apparent difference in occluded sapwood in Douglas-fir and western larch following inoculations suggests that western larch may limit fungal growth more effectively than Douglas-fir. The inability of the fungi to thrive in western larch could contribute to the failure of Douglas-fir beetle attacks on live larch.

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