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**STEM LESIONS AND DIEBACK OF DOUGLAS-FIR SEEDLINGS
WASHINGTON DEPARTMENT OF NATURAL RESOURCES
WEBSTER NURSERY, OLYMPIA, WASHINGTON**

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ABSTRACT

Isolations from stem and branch lesions and stem dieback lesions in 1-0 and 2-0 bare root Douglas-fir seedlings at the Washington Department of Natural Resources Webster Nursery, Olympia, Washington, yielded a wide range of fungi. The most common fungal associates in all three types of samples were *Phoma* spp., particularly *P. eupyrena*. Other fungal associates included *Botrytis cinerea*, *Ulocladium atrum*, four species of *Alternaria*, three species of *Fusarium*, and several unidentified Basidiomycetes. Some lesions were devoid of fungi. Isolations from roots of 2-0 seedlings with stem lesions yielded low levels of *Fusarium* and *Cylindrocarpon* spp. Although most lesions were colonized by fungi, associated organisms may not necessarily have elicited tissue necrosis. Other factors, particularly nutrient imbalances, such as boron deficiency, may have played important roles in formation of seedling lesions. Further work to elucidate differential effects of altering fertilizer regimes on lesions are underway.

INTRODUCTION

The Washington Department of Natural Resources Webster Nursery near

Olympia, Washington produces large numbers of 2-0 coastal Douglas-fir (*Pseudotsuga menziesii* Franco) for reforestation of Washington State lands. Rapidly-growing seedlings at the nursery and are usually devoid of pest problems.

However, seedlings in some beds recently appeared chlorotic, especially within inner seedling rows (figure 1). This pattern of inner row chlorosis was general within particular nursery fields. Another related problem was breakage of the tops of seedlings during their second growing season. This breakage occurred along the main stem and usually several inches above the groundline. Closer examination of many 2-0 seedlings indicated presence of definitive stem (and sometimes branch) lesions (figures 2-4), particularly on seedlings within chlorotic seedling rows. In some cases, stem lesions were associated with definitive necrotic zones where killed tissues were saturated with resin (figures 5-6). Dead needles (figure 5) or branches (figure 6) were sometimes emanating from the necrotic zone. In extreme cases, main stem breakage occurred at the necrotic zone (figure 7). Affected seedlings were generally not grouped together, but were scattered throughout seedbeds. Losses from this problem at the nursery have escalated from about 2-3% during 1999 to nearly 30% in 2001. Only the coastal form of Douglas-fir is affected; interior Douglas-fir (var. *glauca*) are usually not affected. The problem was found among several different Douglas-fir seedlots. Regular regimes of fungicide applications were made on a rotational basis throughout affected seedbeds; chemicals applied included benomyl, iprodione, and a combination of daconil and Cleary's 3336®.

Another related problem on some 2-0 Douglas-fir was terminal leader dieback (figure 8). This resulted in scattered seedlings with necrotic tops; necrosis extended part way down the main stem, but rarely caused seedling mortality.

Similar to those seedlings with stem breakage symptoms, those with leader dieback were scattered throughout seedbeds.

To ascertain possible roles of potentially-pathogen microorganisms in causing stem lesions and dieback of Douglas-fir seedlings, a series of isolations were made from seedlings displaying symptoms.

MATERIALS AND METHODS

Three sets of isolations were made from seedlings displaying stem and bark lesions and dieback symptoms. Eight 1-0 and twelve 2-0 seedlings with stem lesions were sampled. Nine 2-0 seedlings with stem dieback symptoms were also sampled.

Tissue samples were aseptically excised from the edges of lesions by making thin hand sections using a sterile razor blade. Tissue pieces on the edge of lesions (with or without necrosis) were surface sterilized in a 10% bleach solution (05.25 % aqueous sodium hypochlorite), rinsed in sterile, distilled water, blotted dry and aseptically placed on 2% water agar (WA). Several isolations were usually made from each sampled lesion. Plates were incubated in the dark at about 24°C for a minimum of 48 hours. Selected fungi emerging from tissue pieces were transferred to potato dextrose agar for identification. Some isolates of *Fusarium* were also transferred to carnation leaf agar (Fisher et al. 1982), which aided sporulation; *Fusarium* isolates were identified to species using the taxonomy of Nelson et al. (1983). Isolates of *Phoma* and

Cylindrocarpon spp. were identified using the taxonomy of Dorenbosch (1970) and Booth (1966), respectively; *Alternaria* spp. were identified using the taxonomy of Simmons (1981, 1982a, 1982b, 1986). Other fungi were identified using the generic classification scheme of Barnett and Hunter (1998).

Stem dieback lesions were sampled using a similar procedure. Thin hand sections were made at the junction between necrotic and healthy tissues using a sterile razor blade. Tissue pieces were surface sterilized and incubated on WA as described above. Selected emerging fungi were transferred to PDA and identified as described above.

To help elucidate possible roles of root colonizing fungi in initiation of lesions, the twelve 2-0 seedlings sampled for

lesions were also evaluated for level of root colonization by potentially-pathogenic fungi. Root systems were washed thoroughly to remove adhering soil particles. Root pieces approximately 5 mm in length were cut from lateral roots and surface sterilized as described above. Twenty root pieces were randomly selected per seedling and incubated on a selective agar medium for *Fusarium* and closely-related fungi (Komada 1975). Plates were incubated under diurnal cycles of fluorescent light at about 24°C for 7-10 days. Selected emerging fungi were transferred to CLA and PDA for identification using the taxonomy of Nelson et al. (1983) and Booth (1966). Percentage of root pieces colonized by selected fungi were determined.

Figure 1. Rows of slightly chlorotic 2-0 bare root Douglas-fir seedlings – Washington Department of Natural Resources Webster Nursery, Olympia Washington.





Figure 2. 2-0 Douglas-fir seedling with several lesions on its main stem – Washington Department of Natural Resources Webster Nursery, Olympia, Washington.



Figure 3. 2-0 Douglas-fir seedling with stem lesions that have formed callus tissues in their centers – Washington Department of Natural Resources Webster Nursery, Olympia, Washington.



Figure 4. Close-up of a main stem lesion without callus tissue formation on a 2-0 Douglas-fir seedling – Washington Department of Natural Resources Webster Nursery, Olympia, Washington.



Figure 5. Necrotic stem lesion on 2-0 Douglas-fir seedling – Washington Department of Natural Resources Webster Nursery, Olympia, Washington. Note callus tissue being formed within necrotic lesion (arrow).



Figure 6. Necrotic stem lesion on 2-0 Douglas-fir seedling – Washington Department of Natural Resources Webster Nursery, Olympia, Washington. Note necrotic branch in the center of the lesion (arrow).



Figure 7. Necrotic stem lesion where breakage occurred on a 2-0 Douglas-fir seedling – Washington Department of Natural Resources Webster Nursery, Olympia, Washington.



Figure 8. 2-0 Douglas-fir seedling with terminal leader dieback – Washington Department of Natural Resources Webster Nursery, Olympia, Washington.

RESULTS

Isolations made from stem and branch lesions and stem dieback cankers yielded a wide range of fungi (table 1). By far the major group of fungi isolated from 1-0 and 2-0 seedlings were *Phoma* spp. *Botrytis cinerea* Pers. (ex.) Fr. and *Alternaria* spp. were isolated quite frequently from lesions and stem dieback cankers on 2-0 seedlings but not from lesions on 1-0 seedlings. *Ulocladium atrum* G. Preuss (= *Stemphylium atrum*) was isolated from all three seedling types sampled. *Fusarium* spp. were isolated infrequently, and only from branch and stem lesions on 2-0 seedlings. There were also a few unidentified Basidiomycetes (which did not sporulate) that were isolated from

lesions of 2-0 seedlings; these non-sporulating fungi were assigned to Basidiomycetes primarily on the basis of hyphal morphology with typical clamp connections. Several of the lesions sampled from 1-0 seedlings and fewer from 2-0 seedlings did not yield any fungi (table 1).

Five different *Phoma* species were isolated from seedlings (table 2). Overall, the two most common species were *P. eupyrena* Sacc. and *P. herbarum* Westend. Two other species isolated at lower frequencies were *P. glomerata* (Corda) Wollenw. & Hochapfel and *P. pomorum* Thüm. *Phoma chrysanthemicola* Hollós was isolated at relatively low levels from lesions on 2-0 seedlings.

Frequency of occurrence of the four *Alternaria* spp. isolated from seedlings is

summarized in table 3. The most commonly isolated species was *A. alternata* (Fr.) Keissler; other species isolated less frequently and limited to stem and branch lesions on 2-0 seedlings included *A. longissima* Deigh-ton & MacGarvie, *A. crassa* (Sacc.) Rands, and *A. tenuissium* (Kunze ex Pers.) Wilts.

Three species of *Fusarium* were obtained from stem and branch lesions on 2-0 seedlings (table 4). These included *F. avenaceum* (Fr.) Sacc., *F. sporo-trichioides* Sherb., and *F. acuminatum* El. & Ev.

Root isolations from 2-0 seedlings with stem and branch lesions yielded several different fungi that can potentially cause root diseases (table 5). However, colonization levels were quite low. For example, although three species of *Fusarium* were isolated from roots, overall colonization was below 20% which would probably be considered too low to elicit much impact on hosts (James et al. 1991). *Cylindrocarpon* spp. (*C. destructans* (Zins.) Scholten and *C. tenue* Bugn.) were also isolated at low levels. The other isolated fungi were likely saprophytic and probably not capable of causing disease.

Table 1. Fungal colonization of stem and branch lesions and stem dieback cankers on 1-0 and 2-0 Douglas-fir seedlings – Washington Department of Natural Resources Webster Nursery, Olympia, Washington.

Number of Sampled Seedlings	Seedling Type Sampled		
	1-0 (Lesions)	2-0 (Lesions)	2-0 (Stem Dieback)
	8	12	9
Number of Lesions or Cankers Sampled	16	27	9
Total Number of Isolations ¹	16	73	27
Fungal Genera ²			
<i>Phoma</i>	62.5	40.9	81.5
<i>Botrytis</i> ³	0	14.7	7.4
<i>Ulocladium</i> ⁴	6.3	9.1	3.7
<i>Alternaria</i>	0	10.6	7.4
<i>Fusarium</i>	0	9.1	0
Basidiomycetes ⁵	0	6.0	0
None	31.2	9.6	0

¹ Total number of isolations from either stem lesions or stem dieback cankers; several isolations were often made from each sampled lesion or canker.

² Percent of isolations yielding appropriate fungus

³ All isolates were *Botrytis cinerea*

⁴ All isolates were *Ulocladium atrum*

⁵ Unidentified

Table 2. *Phoma* species isolated from stem and branch lesions and stem dieback cankers on 1-0 and 2-0 Douglas-fir seedlings - Washington Department of Natural Resources Webster Nursery, Olympia, Washington¹.

<i>Phoma</i> Species	Seedling Type Sampled		
	1-0 (Lesions)	2-0 (Lesions)	2-0 (Stem Dieback)
<i>P. eupyrena</i>	50.0	29.6	68.2
<i>P. herbarum</i>	30.0	29.6	13.6
<i>P. glomerata</i>	10.0	22.2	9.1
<i>P. pomorum</i>	10.0	7.5	9.1
<i>P. chrysanthemicola</i>	0	11.1	0

¹ Values in table are percent of *Phoma* isolates obtained from lesions or cankers.

Table 3. *Alternaria* species isolated from stem and branch lesions and stem dieback cankers on 1-0 and 2-0 Douglas-fir seedlings - Washington Department of Natural Resources Webster Nursery, Olympia, Washington¹.

<i>Alternaria</i> Species	Seedling Type Sampled	
	2-0 (Lesions)	2-0 (Stem Dieback)
<i>A. alternata</i>	42.8	100.0
<i>A. longissima</i>	28.6	0
<i>A. crassa</i>	14.3	0
<i>A. tenuissimum</i>	14.3	0

¹ Values in table are percent of *Alternaria* isolates obtained from lesions or cankers.

Table 4. *Fusarium* species isolated from stem and branch lesions and stem dieback cankers on 1-0 and 2-0 Douglas-fir seedlings - Washington Department of Natural Resources Webster Nursery, Olympia, Washington¹.

<i>Fusarium</i> Species	2-0 (Lesions)
<i>F. avenaceum</i>	50.0
<i>F. sporotrichioides</i>	33.3
<i>F. acuminatum</i>	16.7

¹ Values in table are percent of *Alternaria* isolates obtained from lesions or cankers.

Table 5. Fungi isolated from roots of 2-0 bare root Douglas-fir seedlings with stem and branch lesions – Washington Department of Natural Resources Webster Nursery, Olympia, Washington¹.

Isolated Fungus	Percent Root Pieces Colonized ²
<i>Fusarium oxysporum</i> (Morphotype 1)	15.4
<i>Fusarium oxysporum</i> (Morphotype 2)	0.8
<i>Fusarium proliferatum</i>	0.4
<i>Fusarium avenaceum</i>	2.1
All <i>Fusarium</i> isolates	18.8
<i>Cylindrocarpon destructans</i>	16.3
<i>Cylindrocarpon tenue</i>	1.7
All <i>Cylindrocarpon</i> isolates	17.9
<i>Trichoderma</i> spp.	25.0
<i>Penicillium</i> spp.	15.0
Other Unidentified Fungi	52.5

¹ Twelve seedlings sampled (same seedlings assayed for fungal colonization of stem and branch lesions – table 1).

² Twenty randomly-selected root pieces sampled per seedling.

DISCUSSION

Many of the stem and branch lesions superficially present on both 1-0 and 2-0 Douglas-fir seedlings at the Webster Nursery were without noticeable necrosis of underlying tissues. These lesions appeared as breaks in epidermal tissues, perhaps in response to rapid terminal and circumferential growth. However, in some other lesions, tissue necrosis was evident. In some cases, definitive callus tissue formation within or on the edge of lesions was evident. Apparently, tissues within lesions may or may not become necrotic and if they do become necrotic, they may or may not callus over. In extreme cases, necrosis occurred and callus tissues did not arrest extension of necrosis around the circumference of stems, resulting in stem breakage.

A wide range of fungal associates was found on stem and branch lesions. There

did not appear to be differentiation of particular fungal taxa within lesions at various stages of necrosis formation, callusing or stem breakage. Apparently the same fungi were present during various stages of lesion development, indicating that these fungal associates may or may not necessarily be involved in lesion progress. Although some of the isolated organisms have been associated with various types of plant diseases, including those on conifer seedlings in nurseries (Jones and Benson 2001), many are considered secondary colonizers of damaged tissues. Without carefully controlled pathogenicity tests to evaluate virulence potential of these fungal taxa under field conditions, it is difficult to assign responsibility of particular organisms in eliciting lesion symptoms seen on seedlings.

The most common group of organisms isolated from all samples was *Phoma*. This form-genus comprises many different taxa which vary widely in their

importance in plant diseases (Domsch et al. 1980; Dorenbosch 1970; James and Hamm 1985). The most commonly encountered species, *P. eupyrena*, is a common soil-borne fungus in forest nursery soils (Dorenbosch 1970; James and Hamm 1985). This species produces thick-walled chlamydospores, usually in chains of varying length, which allow it to survive in soil for prolonged periods (Boerema 1976; Domsch et al. 1980; Dorenbosch 1970). It has frequently been isolated from both healthy and diseased seedling roots (James and Hamm 1985), but is not usually regarded as an important cause of either damping-off or root disease. Rather, its major contribution to seedling disease is its association with tip dieback symptoms of conifer seedlings (James 1979, 1983b, 1983c; James and Cooley 1987; James and Schwandt 1989). *Phoma eupyrena* was initially isolated from "soil cones" that had formed at the base of small, young conifer seedlings that had been partially immersed in light soil due to the action of intense irrigation or rain (James 1979). In this association, the fungus grew from the soil splashed on seedlings and directly attacked young, succulent seedling needle and stem tissues, often resulting in seedling mortality (James 1979; Kliejunas 1984; Kliejunas et al. 1983). This type of disease was especially prominent in nurseries near coastal areas where prolonged period of high humidity and light sandy soils prevailed (Kliejunas 1984; Kliejunas et al. 1983). Losses were often extensive during the first growing season (James 1979). However, as investigations of nursery diseases intensified, *P. eupyrena* was frequently found associated with dieback of stem or branches on bare root seedlings, particularly during the second and

subsequent growing seasons (James 1998). These type of diseases occur on a wide range of conifers, including pine (James and Cooley 1987), spruce (James 1990), and Douglas-fir (James 1998). In some cases, *P. eupyrena* was associated with other fungi, such as *Sphaeropsis sapinea* and *Sirococcus conigenus*, particularly in pine terminal dieback diseases (James and Hamm 1985). However, in other cases, *P. eupyrena* was isolated exclusively and appeared to be the major fungal taxon associated with extensive seedling dieback. Therefore, it appears that this fungus can either be a fairly aggressive pathogen or it may be a common soil-borne saprophyte. There may be genetic differences among isolates related to its ability to elicit disease symptoms. Also, similar genotypes may respond with different levels of aggressiveness on the basis of host stress and susceptibility. It is suspected that *P. eupyrena* was likely important in eliciting stem dieback symptoms on 2-0 Douglas-fir seedlings at the Webster Nursery and may also have been involved in causing necrosis within stem lesions, sometimes leading to stem breakage. The other *Phoma* spp. isolated from seedlings included *P. herbarum*, *P. glomerata*, *P. pomorum* and *P. chrysanthemicola*. All of these species have previously been associated with diseased plants, including conifer seedlings (Boerema 1964, Boerema et al. 1965, James 1983a, 1983b, 1983c, 1984, 1985; James and Hamm 1985). However, their potential for eliciting disease either individually or in combination remains unresolved.

Alternaria spp. are common saprophytes or secondary colonizers of plant tissues (Halfon-Meiri and Rylski 1983; Simmons 1981). *Alternaria alternata*,

which was frequently isolated from Douglas-fir stem lesions and stem dieback tissues, has a history of association with important plant diseases (James and Woo 1987; Jones and Benson 2001). For example, it causes leaf spot diseases on important agricultural and ornamental crops (Belisario et al. 1999; Mortensen et al. 1983) and produces powerful toxins that aid infection of plant tissues (Fuson and Pratt 1988). This versatile fungal species can also act as a hyperparasite on dwarf mistletoe infecting lodgepole pine trees (Mark et al. 1976). It is possible that *A. alternata* contributed to disease initiation within infected Douglas-fir seedlings evaluated in this study.

Fusarium spp. are often associated with several different kinds of conifer seedlings diseases (Bloomberg 1981; James et al. 1991). Although more frequently root pathogens (Bloomberg 1973, 1981; James et al. 1991), *Fusarium* spp. can also elicit above-ground diseases on stems and branches, particularly under conducive growing conditions such as high temperatures and humidity (James 1992, 2003). For example, *F. avenaceum* and *F. sporotrichioides*, two species frequently isolated from 2-0 Douglas-fir stem lesions in the current study, were recently associated with an aggressive disease of container-grown ponderosa pine (James 2003). These fungi frequently sporulated on infected secondary needle fascicles and caused extensive top blight and seedling mortality. Therefore, it is possible that the *Fusarium* isolates from Douglas-fir stem lesions contributed to necrosis associated with some of the lesions. The level of root colonization by *Fusarium* found on Douglas-fir seedlings was quite

low and probably had little effect on above-ground disease initiation.

In conclusion, it seems likely that several different groups of potentially-pathogenic organisms frequently isolated from necrotic Douglas-fir stem and branch tissues were capable of eliciting the disease symptoms. Based on its frequent association with tip blight diseases on a wide variety of conifer species, *P. eupyrena* is likely the best candidate for pathogenicity on these seedlings. However, other *Phoma*, *Alternaria*, and *Fusarium* spp. may also have been involved. These potential pathogens were present on seedlings and within nursery soil despite pre-plant soil fumigation with methyl bromide/chloropicrin and frequent applications of fungicides.

Seedlings may have been predisposed to excessive stem lesion formation by nutrient imbalances or deficiencies present in nursery soil. In particular, boron deficiencies may have contributed to lesion formation because this element is known to activate phenols within plant tissues which may be important in exclusion of potential pathogens. To evaluate the potential of boron to alleviate excessive stem lesion formation, growers plan to initiate treatments with Solubor®, a soluble formulation of boron, on selected seedbeds.

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