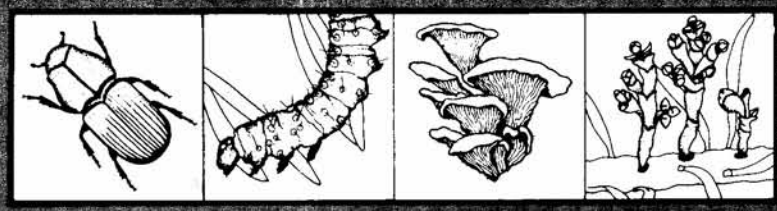
Forest Pest Management



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EVALUATION OF DISEASES OF CONTAINER-GROWN CONIFER SEEDLINGS - COLVILLE CONFEDERATED TRIBAL GREENHOUSE, NESPELEM, WASHINGTON

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ABSTRACT

An evaluation was conducted to determine association levels of potentially pathogenic fungi with diseases of western larch, Engelmann spruce, ponderosa pine and Douglas-fir container-grown seedlings produced during 1991 and 1992 at the Colville tribal greenhouse in Nespelem, WA. *Botrytis cinerea* was commonly colonizing above-ground necrotic tissues of western larch, and *Fusarium* spp. (mostly *F. oxysporum*) was frequently isolated from roots of these seedlings, particularly those grown in 1992. Styroblock containers used for larch seedling production did not harbor large populations of root pathogens. Engelmann spruce seedlings were extensively colonized with root pathogens, particularly *Fusarium* spp. *Fusarium anthophilum* was isolated from most of the sampled 1992 spruce seedlings; this species has not previously been reported on conifer seedlings. Diseased ponderosa pine and Douglas-fir seedlings had roots extensively colonized with *F. proliferatum*. *Phoma glomerata* was also commonly colonizing necrotic tops of pine seedlings. *Cylindrocarpon destructans* was infrequently isolated from roots of many sampled seedlings of all species. Most disease symptoms resulted from root infection and decay caused by several *Fusarium* species. Procedures for reducing damage in the future are discussed.

INTRODUCTION

Production of container-grown conifer seedlings is an important aspect of forest management on lands of the Colville Confederated Tribes in Washington. Several conifer species needed for reforestation on a wide variety of forest sites are produced annually at the tribal greenhouse facility at Nespelem, Washington. Seedling production is sometimes affected by disease occurrence which not only reduces the number of seedlings available for planting, but also seedling quality. Quality directly affects outplanting performance.

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During production of the 1991 and 1992 crops of conifer seedlings at the greenhouse, disease symptoms were detected by growers on several western larch (*Larix occidentalis* Nutt.), Engelmann spruce (*Picea engelmanni* Parry), ponderosa pine (*Pinus ponderosa* Laws.), and Douglas-fir (*Pseudotsuga menziesii* (Mirb.)Franco) seedlings. Top dieback with affected tips often curled over was the most common symptom on western larch (Figure 1). Necrotic needles were usually confined to the top of affected seedlings; much of the necrotic needle complement had fallen prematurely with the exception of the top-most whorl. Western larch seedlings with this type of top dieback usually had few other indications of disease, i. e., their root systems were not extensively decayed or necrotic. Diseased western larch seedlings were located in several pockets within the greenhouse.

Diseased ponderosa pine seedlings were also affected with top dieback (Figure 2). Necrotic needles were often retained on affected seedlings and the top was commonly crooked over. Necrosis often extended into the main stem of diseased seedlings. Roots of affected ponderosa pine had several necrotic lesions and some decay, particularly near root tips. Affected pine seedlings were scattered within the greenhouse.

Foliar necrosis on diseased Engelmann spruce seedlings formed a mosaic throughout the crown (Figure 3). In some cases, necrotic foliage was located within or near the tips of either the main stem or lateral branches. In others, necrosis was prominent in zones below the terminal foliage on branches. Roots of diseased seedlings usually had some decay or evidence of necrotic lesions. Diseased seedlings were spread randomly throughout the greenhouse.

Diseased Douglas-fir seedlings displayed typical wilt symptoms; tips of the main stem and lateral branches were dead with necrotic foliage usually located on tips of seedlings, similar to symptoms produced on diseased ponderosa pine (Figure 2). Roots of diseased Douglas-fir usually had prominent necrotic lesions and decay. Affected seedlings were located randomly throughout the greenhouse.

An evaluation was conducted to quantify associations of pathogenic fungi with diseased seedlings. Non-diseased western larch were also assayed for presence of potential pathogens. In addition, styroblock containers were also sampled for the possibility of harboring potentially pathogenic root pathogens.

MATERIALS AND METHODS

Examples of necrotic foliage from all four conifer species were placed in moist chambers and monitored for emerging fungi. Tissues were thoroughly washed with tap water prior to placement within moist chambers. Emerging fungi were identified to genus using a standard taxonomic guide (Barnett and Hunter 1972). The monograph of Dorenbosch (1970) was used to identify species of *Phoma*.

Since tip dieback is a common root disease symptom in container-grown seedlings (James and others 1988a), seedling roots were analyzed for presence of potential root pathogens. This entailed washing roots thoroughly to dislodge particles of growing media and examining their general condition. A rating system based on percentage of roots with noticeable decay and those with healthy, white root tips was developed (Table 1). The rating system was used to numerically compare relative root system health. After rating each root system, roots were dissected into pieces approximately 5-7 mm in length, surface sterilized in an aqueous solution of sodium hypochlorite (10 percent bleach) and rinsed in sterile water prior to placement on selective agar media. Two media were routinely used to determine presence of potential root pathogens. One was a selective medium for isolation of *Fusarium* spp. and closely related organisms (Komada 1975). The other medium was selective for water mold fungi (*Pythium* and *Phytophthora*) and contained V-8 juice

Figure 1. Top dieback of container-grown western larch seedling from the Colville tribal greenhouse. Necrotic tips were usually colonized with *Botrytis cinerea*.

Figure 2. Top necrosis of container-grown ponderosa pine seedling from the Colville tribal greenhouse. Necrotic needle and stem tissues were frequently colonized with *Phoma glomerata* and root systems colonized with *Fusarium* spp.

Figure 3. Foliar necrosis of container-grown Engelmann spruce seedling from the Colville tribal greenhouse. Necrotic patterns were not restricted to the tips of main stem or lateral branches as was common on wester larch and ponderosa pine. Rather, a mosaic of necrotic needles were common throughout the crown. Diseased seedlings were extensively colonized with *Fusarium* spp.

Table 1. Root system condition rating system used to quantify deterioration of container-grown conifer seedling roots - Colville tribal greenhouse.

Root Decay Description	Numerical Value
None visible	1
Very little decay (less than 5% of roots)	2
Little decay (5-20%)	3
Moderate decay (20-49%)	4
Extensive decay (greater than 50%)	5
White Root Tips	
Greater than 50% of roots with white tips	1
20-50% with white tips	2
5-20% with white tips	3
Less than 5% with white tips	4
No roots with white tips	5
Add values for root decay and white root tips; maximum value = 10 (most deteriorated root system).	

agar amended with the antibiotics pimaricin, rifamycin, ampicillin, and the fungicide pentachloronitrobenzene. Ten randomly selected root pieces from each sampled seedling were placed on these selective media. Plates with Komada's medium were incubated at about 24°C under 12-hour diurnal cycles of cool, fluorescent light for 7-10 days. Fungal isolates emerging from root pieces were identified to genus; selective isolates thought to be potential root pathogens were transferred to both carnation leaf agar (Fisher and others 1982) and potato dextrose agar (PDA) and identifed to species using taxonomic guides of Booth (1966, 1971) and Nelson and others (1983). Plates of V-8 juice agar were incubated at about 24°C in the dark for 3-5 days. Representative isolates growing from root pieces were transferred to PDA; associated water mold fungi were identified using the taxonomic guides of Middleton (1943) and Waterhouse (1968).

From the 1991 crop, 27 diseased western larch, 11 diseased ponderosa pine, and 8 diseased Engelmann spruce seedlings were analyzed. Also from this crop, 13 non-diseased (without foliar necrosis) seedlings were analyzed for presence of potential root pathogens. From the 1992 crop, 10 diseased western larch, 11 diseased Engelmann spruce, and 8 diseased Douglas-fir seedlings were analyzed.

A styroblock container used to grow western larch seedlings was also sampled. This container had both diseased and non-diseased seedlings. After all seedlings were extracted, 13 cells were randomly selected for sampling. Samples consisted of styroblock pieces from the bottoms of cells since this is where most potential root pathogens reside (James and others 1988b). Four pieces (one from each cardinal direction) were aseptically cut from the bottom of each sampled cell. Extracted pieces were placed, inside surface down, on Komada's medium and incubated as described above. Selected isolates were transferred to appropriate media and identified.

Percentage of sampled seedlings and styroblock cells infected with potentially pathogenic fungi were calculated. In addition, colonization intensity was estimated by determining percent of root and styroblock pieces colonized with particular fungi.

RESULTS AND DISCUSSION

Western larch seedlings from both crops were extensively colonized with *Botrytis cinerea* (Fr.) Pers. This fungus was sporulating on necrotic needle and stem tissue which had been placed in moist chambers and incubated for a few days (Figure 4). Roots of diseased and non-diseased seedlings from the 1991 crop were not extensively colonized with either *Fusarium* spp. or *Cylindrocarpon destructans* (Zins.) Scholten (Tables 2 and 3). However, diseased larch seedlings from the 1992 crop were extensively colonized with *Fusarium*, primarily *F. oxsporum* Schlecht. (Table 4). One diseased seedling from the 1992 crop was also infected with *Pythium ultimum* Trow. *Botrytis* was also found on necrotic tips of 1992 western larch and may have combined with *Fusarium* to elicit disease symptoms. Root system deterioration was much greater in the 1992 crop (Table 4), probably due to infection by *Fusarium*.

Engelmann spruce seedlings from both crops had roots infected with *Fusarium* spp. and *C. destructans*. No foliar pathogen was associated with crown dieback symptoms. Root infection levels and root deterioration ratings were higher in the 1992 crop (compare Tables 2 and 4). The most common *Fusarium* spp. isolated from roots of 1992 seedlings was *F. anthophilum* (A. Braun) Wollenw., a species not previously obtained from conifer seedlings in the northern Rocky Mountains. Other species isolated from spruce roots included *F. proliferatum* (Matsushima) Nirenberg, *F. oxysporum*, *F. acuminatum* Ell. & Ev., *F. sporotrichioides* Sherb., and *F. avenaceum* (Fr.) Sacc. All of these species have previously been encountered on container seedlings (James and others 1989b; 1991b), although not all are necessarily pathogens. The role of *F. anthophilum* in causing disease is unknown since pathogenicity of this species on conifer seedlings has not been tested.

Figure 4. *Botrytis cinerea* sporulating from tip of a necrotic western larch needle incubated within a moist chamber. Prominent grayish-colored conidia were produced at the end of black condiophores.

Table 2. Colonization of roots of diseased container-grown conifer seedlings from the Colville tribal greenhouse with selected fungi (1991 crop).

	Conifer Species[1]					
Fusarium spp.[2]	*Western Larch* % Infect.	% Colon.	*Engelmann Spruce* % Infect.	% Colon.	*Ponderosa Pine* % Infect.	% Colon.
FPRO	3.7	1.5	25.0	10.0	72.7	36.4
FOXY	0	0	12.5	5.0	0	0
FACU	11.1	1.1	12.5	5.0	9.1	9.1
All *Fusarium*	14.8	2.6	50.0	20.0	72.7	43.6
Cylindrocarpon destructans	11.1	4.4	12.5	1.3	9.1	0.9

[1] Sample sizes: western larch = 27; Engelmann spruce = 8; ponderosa pine = 11.

% Infect. = percent of sampled seedlings infectged with th eappropriate fungus.

% Colon. = percent of sampled root pieces (10 per seedling) colonized with the appropriate fungus.

Average root system condition rating (see Table 1): western larch = 5.4, Engelmann spruce = 4.5, ponderosa pine = 4.7.

[2] Fusarium species: FPRO - *F. proliferatum*; FOXY = *F. oxysporum*; FACU = *F. acuminatum*.

Table 3. Colonization of non-diseased container-grown western larch seedlings from the Colville tribal greenhouse with selected fungi (1991 crop).[1]

Fusarium Species	Infection Percent	Colonization Percent
F. proliferatum	7.7	6.1
F. sporotrichioides	7.7	1.5
All *Fusarium*	15.4	7.7
Cylindrocarpon destructans	23.1	4.6

[1] Average root system condition rating (see table 1) = 3.1.

Table 4. Root colonization of diseasec ontainer-grown conifer seedlings from the Colville tribal greenhouse with selected fungi (1992 crop).

	Conifer Species[1]					
	Western Larch		*Engelmann Spruce*		*Douglas-fir*	
Fusarium spp.[2]	% Infect.	% Colon.	% Infect.	% Colon.	% Infect.	% Colon.
FPRO	40.0	16.0	0	0	100.0	92.5
FOXY	90.0	71.0	45.4	16.4	0	0
FANT	0	0	81.2	40.9	0	0
FSPO	0	0	9.1	1.8	0	0
FACU	0	0	0	0	12.5	1.3
FAVE	0	0	9.1	1,8	0	0
All *Fusarium*	100.0	85.0	100.0	60.9	100.0	93.4
Cylindrocarpon destructans	10.0	4.0	27.3	5.4	0	0

[1] Sample sizes: Western larch = 10; Engelmann spruce = 11; Douglas-fir = 8.
% Infect. = Percent of sampled seedlings infecterd with the appropriate fungus.
% Colon. = Percent of sampled root peices (10 per seedling) colonized with the appropriate fungus.

Average root system condition rating (see Table 1): western larch = 6.7;
Engelmann spruce = 8.7; Douglas-fir = 6.8.

[2] *Fusarium* species: FPRO = *F. proliferatum*; FOXY = *F. oxysporum*; FANT = *F. anthophilum*; FSPO = *F. sporotarichioides*; FACU = *F. acuminatum*; FAVE = *F. avenaceum*.

Necrotic foliage and tips of diseased ponderosa pine seedlings were commonly colonized with *Phoma glomerata* (Corda) Wollenw. & Hochapf. This fungus readily sporulated on necrotic tissues incubated in moist chambers (Figure 5) and has previously been found on conifer seedlings (James and Hamm 1985), although it is most often encountered on bareroot stock rather than seedlings grown in greenhouses. The fungus has been reported as a pathogen on other plant hosts (Boerema and others 1965; Dorenbosch 1970), but its aggressiveness on conifer seedlings has not been determined. Roots of these pine seedlings were also extensively colonized with *F. proliferatum* (Table 2). This species has previously been commonly isolated from container ponderosa pine with and without disease symptoms (James 1986; James and Gilligan 1988). Relatively little root deterioration was evident on these pine seedlings (Table 2).

Diseased Douglas-fir seedlings from the 1992 crop were also extensively colonized with *F. proliferatum* (Table 4). These seedlings had high levels of root deterioration; no common pathogenic fungi were isolated from necrotic foliar tissues. *Pythium ultimum* was isolated from two of the sampled seedlings, although colonization rates were quite low (less than 15 percent).

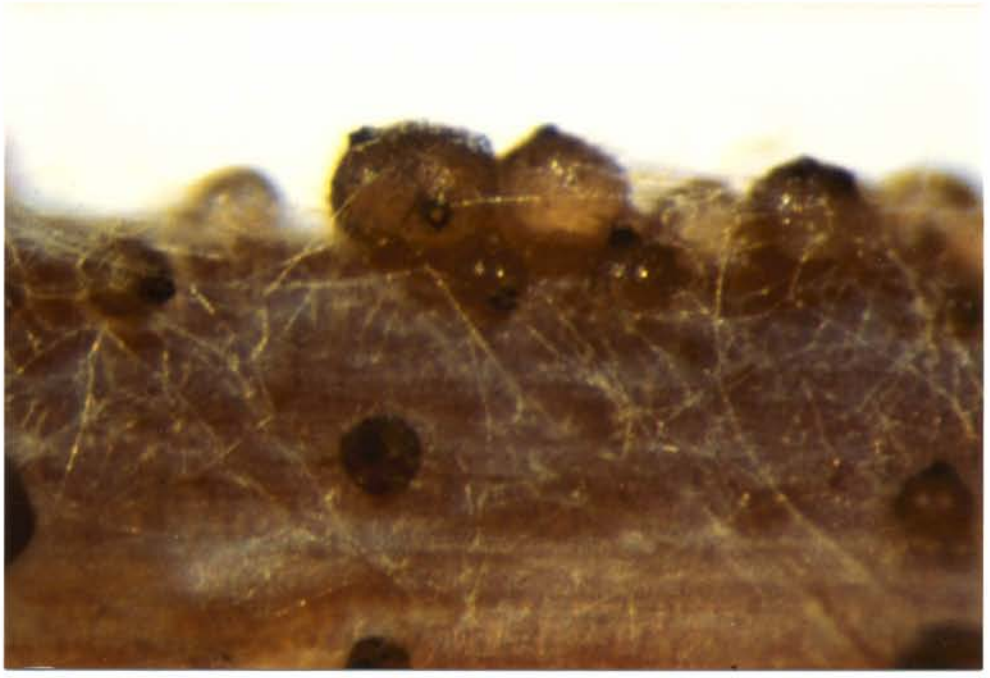

Figure 5. *Phoma glomerata* pycnidia produced superficially on surface of necrotic ponderosa pine needle from diseased seedling grown at the Colville tribal greenhouse.

It appears root disease associated with *Fusarium* spp. was the major cause of above-ground necrosis on the Engelmann spruce and Douglas-fir seedlings examined. It is likely root infection was also important on some western larch seedlings, particularly those from the 1992 crop. Root colonization by *Fusarium* also probably contributed to disease of ponderosa pine, and may have predisposed seedlings to attack by *P. glomerata* which hastened foliar necrosis. *Pythium ultimum*, an important pathogen of many plants including conifer seedlings (James and others 1991a; Middleton 1943), was isolated infrequently from only a few larch and Douglas-fir seedlings. Its role in disease induction was not nearly as important as *Fusarium*.

Although several *Fusarium* spp. were isolated from roots of these conifer seedlings, those of most concern were *F. proliferatum* and *F. oxysporum*. Both these species are known to be pathogenic to conifers, and some isolates may be extremely aggressive (James and others 1989a). *Fusarium proliferatum* is usually encountered more frequently on container seedlings than *F. oxysporum*. However, for the 1992 larch crop, the opposite was true. In any event, high levels of these fungi on seedling roots should be of concern because of their potential to develop rapidly and elicit disease when environmental conditions become conducive (James and others 1991b). Low levels of *Fusarium* are normal and usually of little concern because they may not adversely affect seedling growth or vigor. However, if roots are extensively colonized, seedlings may become diseased, particularly when they are stressed (James and others 1987).

Fusarium anthophilum, frequently isolated from roots of Engelmann spruce seedlings in the 1992 crop (Table 4), is similar taxonomically to *F. proliferatum*. Both species are in the *Fusarium* section *Liseola*, which also includes similar species like *F. moniliforme* Sheldon and *F. subglutinans* (Wollenw. & Reinking) Nelson, Toussoun & Marasas. *Fusarium anthophilum* has not been previously reported as a pathogen on conifer seedlings (Nelson and others 1983). Therefore, its role in causing disease symptoms on seedlings needs to be determined by carefully controlled pathogenicity tests.

Cylindrocarpon destructans was commonly isolated from the roots of many conifer seedlings. This fungus is a common inhabitant of the rhizosphere (Booth 1966) and may be pathogenic under certain circumstances (James 1991). The levels encountered on seedlings from the Colville tribal greenhouse were quite low and probably not important in contributing to disease.

Fusarium spp. were not isolated from styroblock containers used to produce the 1991 crop of western larch (Table 5). This, coupled with the low levels of root infection found on these seedlings, would confirm the reduced role of root colonizing fungi in causing disease symptoms of this crop.

Table 5. Colonization of styroblock containers used to grow diseased and non-diseased western larch seedlings at the Colville tribal greenhouse with selected fungi (1991 crop).

Cells Producing Diseased Seedlings [1]

	Percent Infected	Colonization Intensity [2]
Fusarium spp.	0	0
Cylindrocarpon destructans	7.7	1.9

Cells Producing Non-diseased Seedlings [1]

	Percent Infected	Colonization Intensity [2]
Fusarium proliferatum	7.7	3.8
Cylindrocarpon destructans	7.7	1.9

[1] Sample sizes for both groups = 13 cells

[1] Based on percent of styrofoam pieces sampled at the bottom of each cell (4 per cell) that were colonized with the appropriate fungus.

DISEASE MANAGEMENT

Prevention of diseases of container-grown conifer seedlings is more effective than trying to control diseases once symptoms appear. Reducing pathogen inoculum is an important step in limiting disease impact (James and others 1991b). Sanitation during and between cropping periods is essential to keep inoculum at a minimum. Cleaning interior surfaces of greenhouses with bleach or similar sterilizing solutions between crops is important. Walls, floors, and benches should be included. Removal of organic debris including weeds within and adjacent to greenhouses will help reduce pathogen inoculum. Treating styroblock containers by immersing them in hot water (80°C for 1-3 minutes) will also destroy pathogen inoculum that may be carried over to new seedling crops (James and Woollen 1989). Frequent removal of diseased seedlings from greenhouses is also important to reduce secondary spread of pathogens.

Many root-pathogenic fungi may be introduced into container operations on conifer seed (James 1987). Some growers have reduced such inoculum by treating seed with surface sterilants, such as bleach or hydrogen peroxide, prior to sowing (Dumroese and others 1988). Monitoring pathogen levels on seed might be important, especially on lots with reduced germination, poor seedling establishment, and poor seedling vigor.

Once root disease symptoms appear, it is very difficult to reduce damage by application of pesticides (James and others 1988c). Seedling root systems may be greatly deteriorated by the time above-ground symptoms are discernable (James 1986). Pesticides are usually not very effective as therapeutants of root-diseased seedlings. Therefore, losses may continue even after several chemical applications.

Chemical pesticides are more effective in controlling foliar pathogens such as *Botrytis* (James 1984). However, precautions should be taken because of the propensity of *Botrytis* to rapidly develop resistance to chemical pesticides, particularly if certain chemicals are used repeatedly (Gillman and James 1980). Prevention through sanitation and cultural operations is usually more effective than applications of chemical pesticides in controlling *Botrytis*, as it is with reducing root diseases (James 1984). Of particular importance is to monitor growth and adjust fertilizer and irrigation regimes to keep seedlings at proper height (Dumroese and others 1990). This will help reduce seedling susceptibility to *Botrytis* infection.

Biological control options are not currently available for growers of container-grown conifer seedlings (James and others 1993). Although several commercial products are available, their efficacy on conifer root diseases has not been demonstrated.

Diseases of container-grown conifer seedlings may sometimes be important limiting factors in production. Most growers learn to cope with diseases by instituting integrated pest management procedures including careful monitoring and sanitation (James and others 1990). Crops may be successfully grown with little or no chemical pesticide application (Dumroese and others 1990), which is beneficial from several standpoints including worker safety and environmental contamination. Efforts toward reduced pesticide usage while producing high quality seedlings for reforestation are certainly encouraged.

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