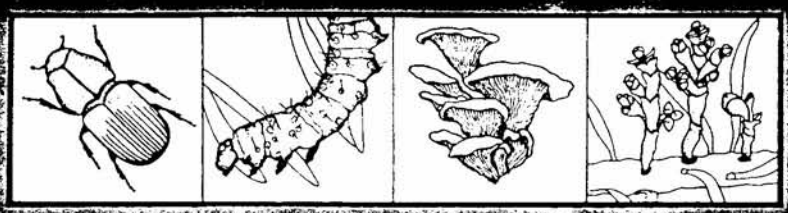
Forest Health Protection



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ROOT DISEASE OF 1-0 BAREROOT SEEDLINGS USDA FOREST SERVICE LUCKY PEAK NURSERY, BOISE, IDAHO

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ABSTRACT

Root disease was common on 1-0 seedlings grown in portions of fields 10 and 12 at the USDA Forest Service Lucky Peak Nursery during 1995. The fields were not fumigated before sowing because soils were too wet during the expected contract period. Ponderosa pine, lodgepole pine, western larch, Douglas-fir, and bitterbrush seedlings were affected to some degree. Damage was most severe on ponderosa pine and western larch in portions of field 10. The most commonly associated pathogen on roots of diseased plants and within soil was *Fusarium oxysporum*. Several seedlings of all species were also infected with *Pythium ultimum*. Alternatives to chemical soil fumigation are currently being evaluated at the nursery.

INTRODUCTION

At the USDA Forest Service Lucky Peak Nursery near Boise, Idaho, bareroot seedlings for reforestation in national forests of the Intermountain Region are usually produced in two growing seasons. Most are conifer forest tree seedlings; however, recently non-conifer plants like bitterbrush are produced to provide plantings for wildlife habitat.

A typical production cycle includes soil fumigation before sowing to reduce impacts of soil-borne pathogens and limit weeds in seedling beds. Fumigation is either performed during late summer and early fall preceding the year of sowing, or in spring a few weeks before planting. To be efficaceous, fumigation must be done when soil is the proper temperature with the right moisture content; these requirements are most easily met in late summer, but often difficult to achieve during spring.

Nursery soil has typically been fumigated by commercial applicators with a mixture of methyl bromide and chloropicrin (MBC)(66 percent and 33 percent, respectively) (Marshall 1985). If properly applied, fumigation greatly reduces or eliminates propagules of pathogenic fungi, weed seeds, and insects within the upper soil profile where seedling roots grow (James 1989; Marshall 1985). Although MBC fumigation is expensive, it is usually cost effective because of increased seedling production and quality.

Methyl bromide is being phased out as a soil fumigant because it depletes stratospheric ozone (NAPIAP 1993). Designated a Class I ozone depleter, methyl bromide cannot be manufactured or used in

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the United States after January 1, 2001. These restrictions are dictated by recent revisions of the U.S. Clean Air Act (James and others 1994b) resulting from international agreements outlined in the Montreal Protocol of 1991 to which the U.S. was a signatory.

Because of imminent methyl bromide restrictions, alternatives to this fumigant are currently being investigated at the nursery. Potential alternatives include fallowing, periodic cultivation, adding organic amendments, biological control, and using other chemical fumigants (James and others 1994b). Tests evaluating non-chemical alternatives were installed at the nursery in 1993; evaluations of an alternative chemical fumigant (dazomet) and biological control were initiated in 1995.

During the spring of 1995, MBC fumigation of several fields before sowing conifer and bitterbrush (*Purshia tridentata* (Pursh) crops was planned. However, an abnormally wet spring made field soil too water-logged for effective fumigation. Once fields began to dry, the commercial MBC applicator was unavailable. Therefore, fields were not fumigated prior to spring sowing.

During the seedling establishment phase, it became apparent that disease levels in several fields were much higher than normal. Disease severity increased during the early weeks of seedling growth, with many affected beds displaying extensive seedling mortality (figures 1 and 2). An evaluation was conducted to determine causes of seedling mortality and estimate levels of potentially pathogenic fungi within affected seedbed soil.

MATERIALS AND METHODS

When seedling mortality became extensive in late spring and early summer of 1995, several symptomatic plants were collected and analyzed for associated organisms. The first sampling in late June included ponderosa pine (*Pinus ponderosa* Laws.), western larch (*Larix occidentalis* Nutt.), lodgepole pine (*Pinus contorta* Dougl.), Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), and bitterbrush. Sampled seedlings were located within either field 10 or 12. All analyzed seedlings displayed typical root disease symptoms, including foliar and root necrosis, foliar chlorosis, and dwarfing. Seedling roots were excised from tops and washed thoroughly under running water to remove soil.

Roots were cut into segments approximately 1 cm long, surface sterilized 1 minute in a 10 percent bleach solution (5.25 percent aqueous sodium hypochlorite), rinsed in sterile, distilled water and placed on two selective media. One medium was selective for *Fusarium* spp. and closely related organisms (Komada 1975). The other medium was selective for *Pythium* spp., *Phytophthora* spp., and associated water-mold fungi. This medium consisted of V-8 juice agar amended with several antibiotics including pimaricin, rifamycin, ampicillin, and the fungicide pentachloronitrobenzene (James and others 1990). Plates of Komada's medium were incubated 7-10 days at about 24°C under diurnal cycles of cool, fluorescent light; those with V-8 juice agar were incubated in the dark at about 24°C for 3-5 days. Fungi emerging from roots were transferred to potato dextrose agar and carnation leaf agar (Fisher and others 1982) for identification. Several taxonomic guides were used to identify associated fungi (Booth 1966; Domsch and others 1980; Dorenbosch 1970; Middleton 1943; Nelson and others 1983).

Thirty-seven ponderosa pine seedlings, either appearing healthy or exhibiting various levels of above-ground root disease symptoms, were sampled in August from the west end of field 10. Seedlings were assayed for root-colonizing fungi as described above.

In August, several soil samples were collected within affected ponderosa pine and western larch seedbeds to determine population levels of *Fusarium* and *Trichoderma* spp. Each sample was a soil core taken to a depth of about 15 cm. Standard soil dilution techniques were employed to determine populations (James and others 1990). Soil was initially sieved to remove rocks, pieces of undecomposed organic matter, and soil aggregations. From each sample, a 5 g subsample was used to calculate oven-dry weight, which provided a standard for comparison. For this determination, samples were dried at about 100°C for at least 24 hours or until sample weight stabilized (all excess moisture removed). For analysis of fungal populations, field-moist soil was used, but fungal populations were reported on an oven-dry weight basis. For assay of *Fusarium* and *Trichoderma* populations, 0.5 g of soil was combined with 100 ml of 0.3 percent water agar and thoroughly mixed. One ml of solution was placed on each of three plates of selective agar medium (Komada 1975) and spread uniformly.

Figure 1. Root disease of 1-0 bareroot ponderosa pine seedlings in field 10, USDA Forest Service Lucky Peak Nursery. Mortality was extensive with few healthy-appearing seedlings.

Figure 2. 1-0 western larch seedlings with root disease, USDA Forest Service Lucky Peak Nursery. Affected seedlings first turned chlorotic, then brown or red.

Plates were incubated 5 days at about 24°C under diurnal cycles of cool, fluorescent light. *Fusarium* and *Trichoderma* colonies were identified by their morphology on the selective medium and populations determined. Ratios of *Trichoderma* to *Fusarium* propagules were calculated to give a rough estimate of potential soil suppressiveness to root pathogens.

RESULTS

Many emerging seedlings in fields 10 and 12 began displaying typical root disease symptoms during late spring and summer (tables 1 and 2). Typical root disease symptoms included foliar chlorosis and necrosis, stunting, and greatly reduced seedling density (figure 1). Seedlings collected in late June with root disease symptoms were readily colonized with potentially pathogenic fungi (table 1). The most frequently isolated fungus from seedling roots was *Fusarium oxysporum* Schlecht.; it was isolated from all sampled ponderosa pine, western larch, and lodgepole pine and most of the Douglas-fir seedlings. *Fusarium oxysporum* was also commonly isolated from roots of bitterbrush seedlings. Other less frequently isolated *Fusarium* spp. included *F. solani* (Mart.) Appel & Wollenw., *F. acuminatum* Ell. & Ev., and *F. sambucinum* Fuckel.

Table 1. Fungal colonization of 1-0 bareroot seedling roots - USDA Forest Service Lucky Peak Nursery (June 1995).

			Percent Seedlings Colonized [1]				
Plant Species	Sample Location	No. Seedlings Sampled	FOXY	Other *Fusarium*	PYUL	BOT	PHEU	CYDE
Ponderosa pine	Field 10	51	100	5.9[2]	19.6	0	0	0
Western larch	Field 10	18	100	11.1[3]	16.7	0	0	0
Lodgepole pine	Field 12	20	100	0	15.0	0	0	0
Douglas-fir	Field 10	19	94.7	0	57.0	0	0	
Bitterbrush	Field 12	36	36.1	5.5[4]	27.8	11.1	27.8	8.3

[1] FOXY = *Fusarium oxysporum*; PYUL = *Pythium ultimum*; BOT = *Botrytis cinerea*; PHEU = *Phoma eupyrena*; CYDE = *Cylindrocarpon destructans*.
[2] Includes *Fusarium solani* and *F. acuminatum*.
[3] Includes *Fusarium sambucinum* and *F. acuminatum*.
[4] Includes *Fusarium solani* and *F. sambucinum*.

Table 2. Root colonization of 1-0 bareroot ponderosa pine seedlings with *Fusarium oxysporum* - USDA Forest Service Lucky Peak Nursery (August 1995).

		Colonization with *Fusarium oxysporum*	
Foliar Symptom Class[1]	No. Seedlings Sampled	Percent Seedling Infection	Percent Root Colonization[2]
Healthy	6	100	94
Chlorotic	12	100	97
Necrotic	19	100	98
All Seedlings	37	100	97

[1] Based on overall appearance of foliage: Healthy=mostly green foliage with no indication of disease; chlorotic=foliage noticeably yellow, but little foliar necrosis; necrotic=foliage typically red or brown.
[2] Based on percent of root pieces (2 or 3 per seedling) colonized with *Fusarium oxysporum*.

Table 3. Soil populations of *Fusarium* and *Trichoderma* within western larch and ponderosa pine seedbeds in field 10 - USDA Forest Service Lucky Peak Nursery (August 1995).

Western Larch Seedbeds				Ponderosa Pine Seedbeds		
	cfu/g[1]				cfu/g[1]	
Sample No.	Fusarium	Trichoderma		Sample No.	Fusarium	Trichoderma
1	2155	5454		1	1141	3289
2	1549	2693		2	940	2215
3	5185	1549		3	671	2081
4	3030	2424		4	3558	940
5	1279	4915		5	2618	873
6	3030	3165				
7	1616	2020				
8	2222	3165				
Average	2508	3173		Average	1786	1880
T/F Ratio[2] = 1.26				T/F Ratio[2] = 1.05		

[1] Colony-forming units per g of oven-dried soil.
[2] ratio of *Trichoderma* to *Fusarium* propagules in the soil.

Table 4. Comparisons of soil populations of *Fusarium* and *Trichoderma* among different fields and sampling dates - USDA Forest Service Lucky Peak Nursery.

			Average cfu/g[1]		
Field	Sample Date	No. Samples	*Fusarium*	*Trichoderma*	T/F Ratio[2]
1	9/95	25	116	469	3.95
4	5/93	25	188	3557	18.90
6	4/95	50	1010	6024	5.96
8	9/95	25	238	5147	21.60
10	8/95	39	2230	2675	1.20
13	5/93	25	122	4893	39.98
14	9/95	25	747	1348	1.80

[1] Colony-forming units per g of oven-dried soil.
[2] Ratio of *Trichoderma* to *Fusarium* propagules in the soil.

Pythium ultimum Trow. was frequently isolated from all seedling species and was especially prevalent on Douglas-fir and bitterbrush roots. Three other potentially pathogenic fungal species were isolated from bitterbrush roots. These included *Botrytis cinerea* Pers. ex. Fr., *Phoma eupyrena* Sacc., and *Cylindrocarpon destructans* (Zins.) Scholten (table 1).

Sampling ponderosa pine seedlings with different levels of above-ground root disease symptoms yielded very high levels of root infection by *F. oxysporum* (table 2). All sampled seedlings were infected, including those that lacked root disease symptoms.

Soil samples collected from seedbeds containing high root-disease-associated seedling mortality yielded very high *Fusarium* populations (table 3). More than 99 percent of the *Fusarium* isolates were *F. oxysporum*; other species found at low levels included *F. acuminatum* and *F. equiseti* (Corda) Sacc. Species of *Trichoderma* were unidentified; populations of these fungi were similar to *Fusarium*. *Trichoderma/Fusarium* (T/F) ratios were low, especially when compared to other fields at the nursery (table 4).

DISCUSSION

Lack of soil fumigation before sowing conifer and bitterbrush crops in nursery fields 10 and 12 resulted in very high levels of root disease during the first growing season. Although damage occurred to several plant species, losses were especially high on western larch and ponderosa pine. Stands of western larch were so severely decimated by disease that few non-diseased seedlings were evident. Disease was scattered throughout seedbeds, although certain portions were more affected than others.

Although several potentially-pathogenic fungi were isolated from roots of diseased seedlings, the fungus most consistently associated with disease was *F. oxysporum*. This species was also the most common *Fusarium* spp. in soil. *Fusarium oxysporum* has a long history of pathogenic behavior on a wide range of plants, including conifer seedlings (Bloomberg 1971; Brownell and Schneider 1985; James and others 1991). It is a rapid colonizer of root cortical cells (Bloomberg 1973; Bloomberg and Trelawny 1970; Katan 1971) and may initiate disease of infected hosts under conducive environmental conditions (Fisher and Toussoun 1983; James and others 1991). Populations of *F. oxysporum* within agricultural fields tend to be very diverse genetically (Correll 1991; Gordon and Okamoto 1992); it is likely this diversity also occurs in forest nursery soil. Populations usually consist of some pathogenic isolates and some that exhibit only saprophytic behavior on plants (Gordon and Okamoto 1992; Sidhu and Webster 1979). Both types readily colonize plant roots; when isolated, pathogenic and saprophytic forms usually look morphologically identical (Brown and Horne 1926; Gerlagh and Blok 1988). Pathogenic potential can be determined by screening isolates for virulence on seedlings, although such procedures often take much time and effort (Hildebrand 1985; James 1996; James and others 1989) and may yield inconsistent results (Freeman and Rodriguez 1993; James and others 1986). Fortunately, new techniques developed to quantify genetic variability within populations of *F. oxysporum* include vegetative compatibility grouping (Elmer and Stephens 1989; Gordon and Okamoto 1991; Leslie 1993), protein analyses (Desjardins and others 1993; Ho and others 1985), and several techniques that differentiate isolates based on nucleic acid polymorphisms (Coddington and others 1987; Kim and others 1992). These procedures help elucidate differential behavior of *F. oxysporum* in soil, but are expensive and require elaborate laboratory facilities and technical expertise. Hopefully, molecular techniques will be more readily available to investigators in the future so that predictions of expected crop damage can be made from soil population assays.

Large populations of *Fusarium* were detected within seedbed soil where disease levels were high. These populations undoubtedly contributed to high disease incidence and were greater than levels recently detected (table 4) and levels found a few years ago in other parts of the nursery (Hoffman and Williams 1988; Marshall 1983, 1985). Relatively high populations of *Trichoderma* may also occur within some soils at the nursery (table 4)(Marshall 1983). Some *Trichoderma* species may be antagonistic toward plant pathogenic fungi, including *Fusarium* (Baker and Cook 1974; Papavizas 1985). These antagonists often produce antibiotics that either inhibit pathogen spore germination or directly kill spores and mycelia (Huang 1992; Kelley 1976). Therefore, high levels of *Trichoderma* and other antagonistic microorganisms are usually desirable to enhance soil suppressiveness to pathogens (Baker 1987; Baker and Cook 1974; Lin and Cook 1979). Although many factors, some of which are nonbiological, contribute to disease suppressiveness in soil (Baker and Cook 1974), population levels of

Trichoderma may be an important contributor to suppressiveness (Kelley 1976; Papavizas 1985). Therefore, the ratio of *Trichoderma* to *Fusarium* propagules in soil (T/F ratio) may be a rough predictor of disease potential in soil. The higher the ratio, the higher the population of *Trichoderma* relative to *Fusarium*. Therefore, less disease potential would be expected in soils with higher T/F ratios. The T/F ratio in field 10 seedbed soil with high disease was quite low when compared to other fields in the nursery. When the T/F ratio is low (near 1.0 or below), higher disease may occur because there may be insufficient antagonists to buffer pathogenic behavior by *Fusarium*.

Pythium ultimum was also commonly isolated from roots of diseased 1-0 seedlings. This soil-borne fungus is a common pathogen of forest tree seedlings (Chen and others 1987; Edmonds and Heather 1973) and a wide range of hosts (Hendrix and Campbell 1973). Although *Pythium* spp. commonly reside in soil at the Lucky Peak Nursery (Hoffman and Williams 1988; James, unpublished), importance of these fungi in eliciting root disease on conifer crops requires investigation. Experience indicates that *Pythium* spp. are usually less important than *Fusarium* spp. as root disease pathogens of conifer seedlings (James 1989; James and others 1991). However, *Pythium* spp. may cause extensive damage, particularly in poorly-drained portions of fields.

Several other fungi isolated from bitterbrush roots are potential pathogens, although they are likely much less important than either *Fusarium* or *Pythium*. *Botrytis cinerea* was located on both roots and foliage of bitterbrush, although it may have been secondary on plant tissues killed by primary pathogens (James 1984). *Phoma eupyrena* is a common soil-inhabiting fungus that may cause tip dieback of conifer seedlings (Dorenbosch 1970; James and Hamm 1985). *Cylindrocarpon destructans*, another common root-inhabiting fungus (Booth 1966), may cause root disease of forest nursery seedlings under certain conditions, particularly in wet and poorly-drained soils (James and others 1994a).

CONCLUSIONS

Without chemical soil fumigation, root disease losses can be extensive in conifer and bitterbrush crops at the Lucky Peak Nursery. It is possible that disease suppressiveness may be enhanced by non-chemical treatment of soil. Particularly, fallowing and/or amending soil with certain organic matter may reduce disease impacts (James and others 1994b). Biological control may also be important in reducing pathogen levels in soil and improving plant resistance to pathogens (Baker and Cook 1974). Although tests are currently underway to test some of these alternatives to chemical soil fumigation, refinement of practices that will keep disease losses within acceptable limits will probably be continuous. An important part of any integrated pest management program will be close monitoring of disease potential so that the most extreme treatments, such as chemical fumigation, will be required in only those areas where high disease impacts are expected. More intensive sampling of potential pathogen populations will be required if integrated pest management is to be successful. Not all plant species respond the same to soil pathogens present at the nursery. Also, it may be more difficult to raise high quality seedlings in certain fields because of disease pressures. Therefore, seedling production guidelines which specifically address expected disease impacts may have to be formulated, at least for certain crop species. These guidelines will require more information on where disease is most important, which crops are most susceptible, and how cropping practices affect disease severity. Disease management will require more effort than in the past when methyl bromide soil fumigation effectively killed all organisms and allowed production of high quality forest tree seedlings without significant disease impacts.

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