NURSERY DISEASE NOTES **USDA FOREST SERVICE**

NORTHERN REGION
FOREST HEALTH PROTECTION

No. 147 **April 2002**

ROOT DISEASE OF 1-0 BARE ROOT PONDEROSA PINE SEEDLINGS LONE PEAK NURSERY, DRAPER, UTAH

R.L. James
Plant Pathologist

ABSTRACT

Bare root 1-0 ponderosa pine seedlings with chlorotic and necrotic foliage in poorly-drained portions of seedbeds at the Lone Peak Nursery, Draper, Utah, were sampled for root colonization by potentially-pathogenic fungi. All sampled seedlings had roots extensively colonized by *Fusarium* spp., especially *F. oxysporum*. *Pythium* and other water mold fungi were isolated infrequently. *Fusarium* either survived pre-plant soil fumigation or re-infested fumigated fields and extensively colonized roots of young seedlings. Stress induced by exposing roots to prolonged periods of high soil moisture probably contributed to disease development. Disease management principles are discussed.

INTRODUCTION

The Lone Peak Nursery near Draper, Utah produces many different tree and shrub species for conservation programs in the state of Utah. Most plants are produced as bare root stock, although container production within greenhouses has been increasing in recent years.

Conifer seedlings comprise an important part of annual production at the nursery. Most conifer seedlings are produced as 2-0 bare root stock. Seedbeds are usually fumigated prior to sowing to reduce potential damage by soilborne pathogens and weeds. The fumigant of choice for many years has been methyl bromide/chloropicrin (MC-33 – 67% methyl bromide, 33% chloropicrin),

which is usually applied in the late summer prior to sowing the following spring. Other soil fumigants (dazomet, Telone®) have been tried periodically at the nursery, but have not been adequately evaluated scientifically.

During the 2001 growing season, bare root 1-0 ponderosa pine (*Pinus ponderosa* Laws.) in a field located near the south border of the nursery displayed typical root disease symptoms (chlorotic and necrotic foliage with associated root decay). Affected seedlings were concentrated in a portion of seedbeds where water drainage was poor (figure 1). Most affected seedlings were located within rows adjacent to irrigation risers. Seedlings farther away from risers, where beds were better drained, were not nearly as affected and generally appeared healthy. An evaluation was conducted to determine importance of potential root pathogens associated with diseased seedlings.

MATERIALS AND METHODS

Ten pine seedlings in various stages of decline (foliage ranged from slightly chlorotic to completely necrotic, i.e., reddish brown), were randomly collected from within affected seedbeds. Seedlings were carefully extracted to include as much of their root system as possible and kept refrigerated during transfer to the laboratory. Seedling roots were washed thoroughly to remove soil particles and aseptically dissected into pieces approximately 5 mm in length. Ten root pieces per seedling were randomly selected, surface sterilized in a 10% bleach solution (0.525% aqueous sodium hypochlorite) and rinsed in sterile water.

Five pieces per seedling were placed in each of two selective agar media: V-8 juice agar amended with pimaricin, rifamycin, ampicillin, and penta-chloronitrobenzene (selective for *Pythium* and other water mold fungi)(James and Beall 1999; James et al. 1994, 1996) and Komada's medium (Komada 1975), which is selective for *Fusarium* and closely-related fungi. Plates with V-8 juice agar were incubated at about 24°C in the dark for 3 days. Isolates of *Pythium* were identified on the basis of their diameter after this time (15-20 mm), feathery margin, and growth within rather than superficially on the agar surface. Selected *Pythium* isolates were transferred to potato dextrose agar (PDA) for identification using the taxonomy of Waterhouse (1968). Plates with Komada's medium were incubated at least 7 days at about 24°C under diurnal cycles of cool, fluorescent light. *Fusarium* colonies were identified by their morphology on the selective medium. Selected *Fusarium* isolates were transferred to carnation leaf agar (Fisher et al. 1982) and PDA for identification using the taxonomy of Nelson et al. (1983). Percent of sampled root pieces colonized by particular fungi were calculated.

Figure 1. Bare root 1-0 ponderosa pine seedlings within poorly-drained portion of seedbeds with root disease symptoms – Lone Peak Nursery, Draper, Utah.

RESULTS AND DISCUSSION

Only three of the ten sampled seedlings had roots colonized by *Pythium* spp. (table 1). No other water mold fungi, i.e., *Phytophthora* spp., were isolated from seedling roots. All *Pythium* isolates were identified as *P. irregulare* Buisman. The three infected seedlings had relatively low levels of these fungi colonizing their roots. In contrast, all sampled seedlings were extensively colonized by *Fusarium* spp. (table 1). Nine of the 10 seedlings had all their sampled roots colonized by these potential pathogens. The most common *Fusarium* species isolated from roots was *F. oxysporum* Schlecht. This species is a very important soilborne pathogen in bare root nurseries in western North America (Bloomberg 1976; Bloomberg and Lock 1972; James et al. 1991b; Smith 1967). It readily infects and colonize cortical root cells of a wide range of susceptible plants (James et al. 1991). Soil populations of

F. oxysporum normally contain both pathogenic and non-pathogenic isolates (Appel and Gordon 1995; Bloomberg and Lock 1972; Gordon and Martyn 1997; Gordon and Okamoto 1992a). Most *F. oxysporum* isolates are morphologically very similar (James et al. 1991b; Nelson et al. 1983); pathogenic strains can only be identified on the basis of molecular genetic tests (Appel and Gordon 1995; Gordon and Okamoto 1992b, 1992c; Kistler 1997; Kistler et al. 1991) or through controlled inoculation tests (James et al. 1989, 2000). Because of this, it is often very difficult to predict disease potential from only soil population assays. Most *F. oxysporum* isolates capable of eliciting disease on conifer seedlings reside either within existing plant roots or on organic matter within soil (such as residual seedling roots from previous crops)(James and Gilligan 1988; James and Perez 1999a).

Four other *Fusarium* spp. were isolated from seedling roots, but at much lower levels than *F. oxysporum* (table 1). All of these species are good soil saprophytes that tend to live on dead, organic matter. However, they are capable of colonizing seedling roots and some may be able to elicit disease symptoms under certain conditions. For example, *F. acuminatum* Ell. & Ev. and *F. sporotrichioides* Sherb. are both capable of causing disease on young conifer germinants under controlled laboratory conditions (James 2000; James and Perez 1999b). However, under normal nursery conditions, they are probably weak pathogens that are only able to initiate disease when seedlings are severely stressed.

The other fungal group isolated from seedling roots was *Trichoderma*. These fungi are common soil inhabitants and are often isolated from conifer seedling roots (James et al. 1996). They are generally considered saprophytic, but some isolates may be antagonistic toward pathogens such as *Fusarium* and *Pythium* (Papavizas 1985). Generally, population levels of *Trichoderma* spp. are inversely proportional to *Fusarium* spp. within nursery soil or on seedling roots (James 2002; James and Perez 1999a). In the current evaluation, *Trichoderma* spp. were isolated from only half the sampled pine seedlings and, with two exceptions, at relatively low levels (table 1).

Based on isolation results, it appeared that ponderosa pine seedling decline and death was highly correlated with root colonization by *Fusarium* spp., particularly *F. oxysporum*. Despite being in portions of seedbeds with water drainage problems, *Pythium* and other water mold pathogens were not consistently associated with diseased seedlings. *Fusarium* root colonization may be quite common on bare root conifer seedlings without inducing disease symptoms (Bloomberg 1976; James and Gilligan 1988; James et al. 1991b). However, when seedlings become stressed, e.g., high temperatures or excessive moisture, disease can occur (Bloomberg 1976; James et al. 1989, 1991b).

Normally, pre-plant soil fumigation reduces potential for soilborne diseases by eliminating or greatly reducing populations of potential pathogens (James 1989; James et al. 1996; Stone et al. 1997). However, if portions of fields are not fumigated, such as along

irrigation risers, fumigated fields can be readily re-infested by pathogens from non-fumigated areas (James et al. 1991b; Vaartaja 1967). Under such conditions, disease potential can be higher than in fields without fumigation (James 1989; James et al. 1991a). Therefore, entire fields should be fumigated; irrigation risers should be removed prior to fumigation (James et al. 1991a). Whenever possible, it is best not to have fumigated and non-fumigated fields adjacent to each other because of potential problems of re-infesting fumigated fields. Also, areas with water drainage problems should be deep ripped to improve drainage. If drainage problems persist, such areas should be planted with species that can tolerate high moisture levels without becoming stressed to the point of being damaged by resident soil fungi.

When groups of diseased seedlings are discovered, fungicide treatments may not always effectively reduce losses. If water mold fungi are the major pathogens, soil drenches with metalaxyl may help control disease (Afek et al. 1990; Brantner and Windels 1998; Bruin and Edgington 1982). However, fungicide soil drenches are generally ineffective against *Fusarium*-caused diseases (Bloomberg and Lock 1972; James et al. 1991b).

Preventing soilborne diseases rather than relying on therapeutic treatments is usually best. Pre-plant soil fumigation is usually effective (James 1989), but sometimes disease occurs despite fumigation (James and Beall 1999; James et al. 1991a, 1994; Stone et al. 1997). It is always important to properly diagnose the pathogens causing disease, since treatment effectiveness is greatly enhanced with proper diagnosis. Growing pathogen-tolerant crops in particular fields may be necessary to reduce disease impacts. Minimal selective use of pesticides is usually preferred.

Table 1. Colonization or roots of diseased ponderosa pine seedlings with selected fungi – Lone Peak Nursery, Draper, Utah[1].

Seedling Number	*Fusarium*[2]						*Pythium*	*Trichoderma*
	FOXY	FACU	FEQU	FSAM	FSPO	ALL		
1	40	60	20	40	0	100	0	0
2	100	0	0	0	0	100	20	0
3	20	60	20	0	0	100	0	20
4	20	40	20	0	40	100	0	0
5	100	0	0	0	0	100	0	0
6	80	0	20	0	0	100	0	40
7	60	0	0	0	0	60	0	100
8	100	0	0	0	0	100	20	0
9	60	60	0	0	0	100	0	40
10	40	40	20	0	0	100	60	100
Average	62	26	10	4	4	96	10	30

[1] Values in table are percent of sampled root pieces (5 each sampled per seedling for *Fusarium* and *Pythium*) colonized by the appropriate fungus.

[2] FOXY = *F. oxysporum*; FACU = *F. acuminatum*; FEQU = *F. equiseti*; FSAM = *F. sambucinum*; FSPO = *F. sporotrichioides*.

LITERATURE CITED

Afek, U., J.A. Menge and E.L.V. Johnson. 1990. Effect of *Pythium ultimum* and metalaxyl treatments on root length and mycorrhizal colonization of cotton, onion, and pepper. Plant Disease 74:117-120.

Appel, D.J. and T.R. Gordon. 1995. Intraspecific variation within populations of *Fusarium oxysporum* based on RFLP analysis of the intergenic spacer region of the DNA. Experimental Mycology 19:120-128.

Bloomberg, W.J. 1976. Distribution and pathogenicity of *Fusarium oxysporum* in a forest nursery soil. Phytopathology 66:1090-1092.

Bloomberg, W.J. and W. Lock. 1972. Strain differences in *Fusarium oxysporum* causing diseases of Douglas-fir seedlings. Phytopathology 62:481-485.

Brantner, J.R. and C.E. Windels. 1998. Variability in sensitivity to metalaxyl in vitro, pathogenicity, and control of *Pythium* spp. on sugar beet. Plant Disease 82:896-899.

Bruin, G.C.A. and L.V. Edgington. 1982. Induction of fungal resistance to metalaxyl by ultraviolet radiation. Phytopathology 72:476-480.

Fisher, N.L., L.W. Burgess, T.A. Toussoun and P.E. Nelson. 1982. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. Phytopathology 72:151-153.

Gordon, T.R. and R.D. Martyn. 1997. The evolutionary biology of *Fusarium oxysporum*. Annual Review of Phytopathology 35:111-125.

Gordon, T.R. and D. Okamoto. 1992a. Population structure and the relationship between pathogenic and nonpathogenic strains of *Fusarium oxysporum*. Phytopathology 82:73-77.

Gordon, T.R. and D. Okamoto. 1992b. Variation in mitochondrial DNA among vegetatively compatible isolates of *Fusarium oxysporum*. Experimental Mycology 16:245-250.

Gordon, T.R. and D. Okamoto. 1992c. Variation within and between populations of *Fusarium oxysporum* based on vegetative compatibility and mitochondrial DNA. Canadian Journal of Botany 70:1211-1217.

James, R.L. 1989. Effects of fumigation on soil pathogens and beneficial microorganisms. *In*: Landis, T.D. (tech. coord.). Proceedings: Intermountain Forest Nursery Association Meeting. USDA Forest Service, Rocky Mountain Research Station. General Technical Report RM-184. pp. 29-34.

James, R.L. 2000. Pathogenic characteristics of *Fusarium acuminatum* isolated from inland Pacific Northwest nurseries. USDA Forest Service, Northern Region, Forest Health Protection. Report 00-16. 8p.

James, R.L. 2002. Effects of preplant soil treatments on *Fusarium* and

Trichoderma populations and fungal root colonization of 1-0 nondiseased ponderosa pine seedlings - USDA Forest Service Lucky Peak Nursery, Boise, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Report 02-3. 9p.

James, R.L., W. Antrobius and C.J. Gilligan. 1991a. Dwarfing of bareroot western larch seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Report 91-7. 14p.

James, R.L. and K. Beall. 1999. An evaluation of the effects of dazomet on soil-borne diseases and conifer seedling production - USDA Forest Service Lucky Peak Nursery, Boise, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Report 99-9. 15p.

James, R.L., R.K. Dumroese and D.L. Wenny. 1991b. *Fusarium* diseases of conifer seedlings. *In*: Sutherland, J.R. and S.G. Glover (eds.). Proceedings of the First Meeting of IUFRO Working Party S2.07-09 (Diseases and Insects in Forest Nurseries). Forestry Canada, Pacific Forestry Centre, Information Report BC-X-331. pp. 181-190.

James, R.L., R.K. Dumroese, C.J. Gilligan and D.L. Wenny. 1989b. Pathogenicity of *Fusarium* isolates from Douglas-fir seed and container-grown seedlings. Idaho Forest, Wildlife and Range Experiment Station Bulletin No. 52. 10p.

James, R.L. and C.J. Gilligan. 1988b. Occurrence of *Fusarium* on the roots of nondiseased bareroot Douglas-fir seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Report 88-12. 4p.

James, R.L., D.M. Hildebrand, S.J. Frankel, M.M. Cram and J.G. O'Brien. 1994. Alternative technologies for management of soilborne diseases in bareroot forest nurseries in the United States. *In*: Perrin, R. and J.R. Sutherland (eds.). Diseases and Insects in Forest Nurseries. Dijon, France, October 3-10, 1993. Institut National de la Recherche Agronominque. Les Colloques No. 68. pp. 237-246.

James, R.L., D.S. Page-Dumroese, S.K. Kimball and S. Omi. 1996. Effects of *Brassica* cover crop, organic amendment, fallowing, and soil fumigation on production of bareroot Douglas-fir seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Report 96-5. 16p.

James, R.L. and R. Perez. 1999a. Fungal colonization of residual conifer seedling roots in soil - USDA Forest Service Lucky Peak Nursery, Boise, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Report 99-10. 13p.

James, R.L. and R. Perez. 1999b. Pathogenic characteristics of *Fusarium sporotrichioides* isolated from inland Pacific Northwest forest nurseries. USDA Forest Service, Northern Region, Forest Health Protection. Report 99-8. 11p.

James, R.L., R. Perez, R.K. Dumroese and D.L. Wenny. 2000. Virulence of *Fusarium oxysporum* on Douglas-fir germinants: comparison of isolates from nursery soil and roots of healthy and diseased seedlings. *In*: Lilja, A. and J.R. Sutherland (eds.). Proceedings of the 4th Meeing of IUFRO Working Party 7.03.04 – Diseases and Insects in Forest Nurseries. Finnish Forest Research Institute. Research Papers 781. pp.49-64.

Kistler, H.C. 1997. Genetic diversity in the plant pathogenic fungus *Fusarium oxysporum*. Phytopathology 87:474-479.

Kistler, H.C., E.A. Momol and U. Benny. 1991. Repetitive genomic sequences for determining relatedness among strains of *Fusarium oxysporum*. Phytopathology 81:331-336.

Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Review of Plant Protection Research (Japan) 8:114-125.

Nelson, P.E., T.A. Toussoun and W.F.O. Marasas. 1983. *Fusarium* species: an illustrated manual for identification. The Pennsylvania State University Press, University Park. 193p.

Papavizas, G.C. 1985. *Trichoderma* and *Gliocladium*: biology, ecology, and potential for biocontrol. Annual Review of Phytopathology 23:23-54.

Smith, R.S. 1967. Decline of *Fusarium oxysporum* in roots of *Pinus lambertiana* seedlings transplanted into forest soils. Phytopathology 57:1265.

Stone, J.K., D. Hildebrand, R.L. James and S.J. Frankel. 1997. Alternatives to chemical fumigation in bareroot forest nurseries: effects on pathogen levels and seedling density, mortality and quality. *In*: James, R.L. (ed.). Proceedings of the Third Meeting of IUFRO Working Party S7.03-04 (Diseases and Insects in Forest Nurseries). USDA Forest Service, Northern Region, Forest Health Protection. Report 97-4. pp. 59-69.

Vaartaja, O. 1967. Reinfestation of sterilized nursery seedbeds by fungi. Canadian Journal of Microbiology 13:771-776.

Waterhouse, G.M. 1968. The genus *Pythium* Pringsheim. Commonwealth Mycological Institute, Kew, Surrey, England. Mycological Papers No. 110. 70p.

R.L. James is Plant Pathologist, USDA Forest Service, Northern Region, Forest Health Protection. Address: USDA Forest Service, 3815 Schreiber Way, Coeur d'Alene, ID 83814; email rjames@fs.fed.us.