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FUSARIUM ROOT DISEASE OF BARE ROOT 1-0 WESTERN WHITE PINE AND DOUGLAS-FIR SEEDLINGS USDA FOREST SERVICE NURSERY, COEUR D'ALENE, IDAHO

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ABSTRACT

Isolations from roots of scattered diseased 1-0 western white pine and Douglas-fir bare root seedlings at the USDA Forest Service Nursery, Coeur d'Alene, Idaho yielded high levels of *Fusarium*, particularly *F. oxysporum*. Fairly high levels of *F. sporotrichioides* and *F. acuminatum* were also isolated from roots of diseased white pine seedlings. Since all seedlings were grown in soil previously fumigated with dazomet, it is likely that much pathogen inoculum occurred on sown seeds, particular in white pine beds where resident levels of soil *Fusarium* populations were low. It is important to monitor commonly-used seedlots for seed-borne pathogen potential and subject highly contaminated lots to surface sterilization treatments.

INTRODUCTION

Root diseases caused by *Fusarium* spp. are probably the most important production-limiting diseases at the USDA Forest Service Nursery in Coeur d'Alene, Idaho. These diseases are especially important during the first growing season on bare root conifer seedlings (James 2001, 2002). Seed-borne *Fusarium* is often associated with damping-off losses during seed germination and seedling establishment (James 1986, 1987) and soil-borne inoculum is important in causing both damping-off and root diseases of older

seedlings (James et al. 1991). Generally, seedlings surviving the first growing season are usually not adversely affected during their second growing season. Although all conifer species may be affected by *Fusarium* diseases, Douglas-fir (*Pseudotsuga menziesii* Franco var. *glauca* [Mayr.] Sudw.) and western white pine (*Pinus monticola* Dougl.) are quite susceptible and often severely impacted (James et al. 2004a, 2004b).

Near the end of the first growing season, stands of bare root western white pine seedlings were extremely sparse (figure 1), with recently-killed seedlings interspersed randomly throughout stands (figure 2). Intermixed within seedling stands were extensive weed populations (figure 3). At the same time, in an adjacent field, extensive mortality of 1-0 Douglas-fir seedlings was evident (figure 4). Recently-killed seedlings were interspersed with healthy-appearing seedlings.

Since similar disease had previously been reported at the Coeur d'Alene Nursery (James 1993, 2000; James et al. 2004a, 2004b), it was suspected that root pathogens were probably responsible for mortality and likely contributed to the sparse seedling stands. An evaluation was conducted to confirm identity of associated organisms and determine soil populations of potential pathogens within affected areas.

MATERIALS AND METHODS

Three sets of isolations were made: from diseased and healthy-appearing seedlings, from soil surrounding the roots of diseased seedlings, and from soil obtained from the rhizosphere of diseased seedlings. All isolations were made onto a selective agar medium for *Fusarium* and closely-related fungi (Komada 1975). Isolations were made from roots of seven and eleven recently-killed Douglas-fir and western white pine seedlings, respectively. Similar isolations were made from two and three healthy-appearing Douglas-fir and western white pine seedlings, respectively. Selected seedlings were carefully extracted from soil to include as much of their root systems as possible. Seedling roots were carefully washed under running tap water and dissected into pieces approximately 5 mm in length; the entire attached root system of each seedling was sampled (10-21 root pieces sampled per seedling). All root pieces were surface sterilized in an aqueous solution of 0.525% sodium hypochlorite (10% common household bleach), rinsed in sterile distilled water, blotted dry, and placed on the selective agar medium. Fifteen root pieces were placed on agar within each petri plate. Roots were incubated at about 24°C under diurnal cycles of cool, fluorescent light for 7 days. Associated fungi were identified to genus based on spore morphology (Barnett and Hunter 1998).

Selected colonies of suspected pathogens were transferred to carnation leaf (Fisher et al. 1982) and potato dextrose agar (PDA) for identification. *Fusarium* species were identified using the taxonomy of Nelson et al. (1983). The percent of sampled seedlings infected and the extent of root system colonized by specific potential pathogens were calculated.

Figure 1. Sparse stands of 1-0 bare root western white pine seedlings - USDA Forest Service Coeur d'Alene Nursery, Idaho.

Figure 2. Mortality of 1-0 bare root western white pine seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho. Affected seedlings were randomly scattered throughout seedbeds.

Figure 3. Recently-killed 1-0 bare root western white pine seedling surrounding by high populations of groundcover weeds - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Figure 4. Several recently-killed 1-0 bare root Douglas-fir seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Soil samples were collected adjacent to diseased seedlings; three and four samples were obtained in Douglas-fir and western white pine beds, respectively. Samples were collected to a depth of about 20 cm using a standard soil probe (about 5 mm in diameter).

Each soil sample consisted of cores from four probe sub-samples (collected at the four cardinal directions around a diseased seedling); the sub-samples were collated and mixed together to form a larger sample. Soil was placed into plastic bags, kept refrigerated, and transported to the laboratory for analysis.

Standard dilutions (Hildebrand and Dinkel 1988; James and Beall 1999; James et al. 1990, 1996) were conducted on the soil samples. Soil from each sample was initially sieved (2 mm sieve) to remove rocks, pieces of organic matter, and soil aggregates. From each sample, an approximate 5 g subsample was oven-dried at about 100°C for at least 24 hours until sample weight had stabilized. Oven-dry weight was then calculated to provide a standard for sample comparisons. For assays of *Fusarium* and *Trichoderma* populations, 0.05 g of field-moist soil was combined with 10 ml of 0.3 percent water agar and thoroughly mixed. One ml of solution was placed on each of three plates of the selective agar medium and spread uniformly. Plates were incubated as described above. *Fusarium* and *Trichoderma* colonies were identified by their morphology on the selective medium; populations were calculated as number of colony-forming units (cfu) per g of oven-dried soil. Selected *Fusarium* isolates were identified as described above.

Soil adhering to the roots of diseased seedlings (rhizosphere soil) was also assayed. Roots from three each of Douglas-fir and western white pine seedlings displaying above-ground disease symptoms (necrotic foliage) were immersed in 100 ml of sterile, distilled water and thoroughly agitated to remove rhizosphere soil. The solution was thoroughly blended and 1 ml of the solution was placed on three plates of selective agar medium (for each sample) and spread uniformly. Plates were incubated as described above and the average number of *Fusarium* colonies per ml of rhizosphere soil solution was calculated.

RESULTS AND DISCUSSION

All sampled diseased western white pine and Douglas-fir seedlings were infected with *Fusarium* spp. (table 1). Most healthy-appearing seedlings were likewise infected. Root systems of diseased seedlings were extensively colonized by these potential pathogens. However, root systems of healthy white pine were much less colonized by *Fusarium* spp. compared to those of healthy Douglas-fir.

Soil surrounding roots of diseased Douglas-fir seedlings contained fairly high levels of *Fusarium* propagules (table 2), whereas those near white pine seedlings had much lower populations. Higher *Fusarium* levels generally corresponded to lower *Trichoderma* populations, resulting in lower ratios of *Trichoderma* to *Fusarium* populations. Lower ratios may denote decreased potential for disease suppressiveness by

species of *Trichoderma* which can potentially exert biological control (James and Beall 1999; Papavizas 1985).

Seven different *Fusarium* species were isolated from either roots of white pine and Douglas-fir seedlings or adjacent soil (table 3). By far the most commonly-associated species was *F. oxysporum* Schlecht.. Three different morphotypes of this large, genetically-diverse species were encountered. Morphotypes were separated on the basis of colony morphology on PDA, i.e., extent of aerial or suppressed mycelium (pionnotal), pigmentation, and sporodochial production. Isolates of *Fusarium oxysporum* may exhibit a wide range of pathogenic potential on conifer seedlings (Gordon and Martyn 1997; James et al. 1991; Nelson et al. 1983). Because pathogenic and non-pathogenic strains appear similar morphologically (Bao et al. 2002; Gordon and Martyn 1997), it is unknown what proportion of the isolates obtained in this investigation were pathogens. It is suspected that most isolates from roots of seedlings exhibiting disease symptoms were pathogens, although pathogenic strains may occur on roots of seedlings without above-ground disease symptoms (James et al. 2000). Recent genetic work (Stewart et al. 2004) indicated that pathogenic and non-pathogenic *F. oxysporum* strains from forest nurseries may be separated on the basis of molecular genetic markers. If further work confirms this, rapid analysis of pathogenic populations within nurseries may be possible in the future.

Table 1. *Fusarium* infection and colonization of roots from diseased and healthy-appearing 1-0 bare root western white pine and Douglas-fir seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Sample Category	Western White Pine		Douglas-fir	
	Diseased	Healthy	Diseased	Healthy
Number of Seedlings Sampled	11	3	7	2
Percent of Seedlings Infected[1]	100	67	100	100
Number of Root Pieces Sampled	155	55	105	37
Percent of Root Pieces Colonized[1]	87.1	16.4	97.1	100

[1] Infected and colonized by *Fusarium* spp.

Table 2. Populations of *Fusarium* and *Trichoderma* spp. within nursery soil adjacent to diseased western white pine and Douglas-fir seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Fungal Genera	Soil Populations [cfu/g]	
	Western White Pine	Douglas-fir
Fusarium	154	1519
Trichoderma	291	91
T/F Ratio[1]	1.89	0.06

[1] Ratio of *Trichoderma* to *Fusarium* populations; higher numbers denote greater potential for disease suppression by *Trichoderma* spp.

Table 3. *Fusarium* species colonizing 1-0 bare root western white pine and Douglas-fir seedlings and adjacent soil - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Fusarium Species[1]	Western White Pine[2]		Douglas-fir[2]		Soil[3]
	Diseased	Healthy	Diseased	Healthy	
FOXY-1	36.8	0	80.9	89.2	67.1
FOXY-2	5.2	0	30.5	54.0	10.5
FOXY-3	3.9	0	11.4	10.8	6.6
ALL FOXY	45.8	0	85.4	100.0	84.2
FAVE	0	0	9.5	0	11.8
FACU	23.9	5.4	5.7	0	4.0
FSPO	27.7	10.9	0	0	0
FCUL	0	0	5.7	0	0
FEQU	0.6	0	0	0	0
FSAM	0	7.3	0	0	0

[1] FOXY = *Fusarium oxysporum* [numbers denote different morphotypes]; FAVE = *F. avenaceum*; FACU = *F. acuminatum*; FSPO = *F. sporotrichioides*; FCUL = *F. culmorum*; FEQU = *F. equiseti*; FSAM = *F. sambucinum*.
[2] Percent of sampled root pieces colonized by particular *Fusarium* species.
[3] Percent of *Fusarium* isolates obtained from all soil samples.

All the *Fusarium* species encountered have previously been isolated at the Coeur d'Alene Nursery (James et al. 1989). They are commonly found within nursery soil (James 2000b, 2004; James and Perez 1999; James et al. 1996, 2004a, 2004b) as well as on conifer seedling roots (James and Perez 1999; James et al. 2004a, 2004b). *Fusarium sporotrichioides* Sherb. and *F. acuminatum* Ell. & Ev. were isolated at relatively high levels from the roots of diseased white pine seedlings (table 3). These species probably are saprophytes and may not be capable of eliciting seedling disease unless hosts are

extensively stressed (James 2000a; James and Perez 1999). However, it is possible that one or more of these species may affect disease development by influencing the ability of pathogenic strains of *F. oxysporum* to cause disease (Hoff et al. 2004; James et al. 1991). Some *Fusarium* strains are root-colonizing endophytes of conifer seedlings and may be present without adversely affecting host physiology (Bloomberg 1966; Hoff et al. 2004). They would be routinely isolated from surface-sterilized roots (Hoff et al. 2004; Stone et al. 2000), but not necessarily act as pathogens. Further work is required to elucidate their potential roles in seedling disease etiology.

Soil within which diseased seedlings were found had been fumigated with dazomet (Basamid®) prior to sowing. Therefore, most potentially-pathogenic fungi had probably been killed by fumigation. Pathogens isolated from diseased seedling roots were most likely introduced on contaminated seed.

Most pathogens on conifer seed are located externally on seedcoats as passive contaminants (James 1986, 1987). If they can be satisfactorily removed, disease potential to young seedlings is greatly decreased (James 1986). Pre- or post-stratification running water rinses for a minimum of 48 hours can greatly reduce seed surface contamination (Campbell and Landis 1990; Dumroese et al. 1988; James 1986, 1987), particularly if seeds are agitated during rinsing. However, highly-contaminated seeds may need treatment with surface sterilants to adequately remove most pathogen propagules (Campbell and Landis 1990; James 1986, 1987). Chemical treatments are often operationally used on conifer seeds; the two most effective are sodium hypochlorite (bleach) and hydrogen peroxide. Bleach treatment prior to running water rinses has become standard for seeds of many conifer species at some nurseries (Wenny and Dumroese 1987). Hydrogen peroxide is used less frequently, but can be effective as long as chemical concentrations are insufficient to elicit phytotoxicity on young seedling germinants (Barnett 1976; Carter and Jones 1962). Some growers have treated seeds with fungicides (Berbee et al. 1953; Bloomberg and Trelawny 1970). Although results have varied, most fungicides are usually not recommended for seed treatment because of their high potential to reduce germination and cause damage to young, succulent germinants (Cayford and Waldon 1967; Fraser and Adams 1980).

In most cases, conifer seedlots are not routinely screened in the United States to determine level of contamination with potential pathogens. Such tests are becoming common elsewhere, particularly in Canada and parts of Europe. Testing widely-used seedlots for presence of pathogens will help growers select the best lots for sowing as well as concentrate their treatment efforts on those lots exhibiting the greatest contamination (Campbell and Landis 1990). Reducing seedborne pathogens will greatly improve seed germination, seedling establishment, and performance in both bare root and container nurseries.

LITERATURE CITED

Bao, J.R., D.R. Fravel, N.R. O'Neill, G. Lazarovits and P. van Berkum. 2002. Genetic analysis of pathogenic and nonpathogenic *Fusarium oxysporum* from tomato plants. Canadian Journal of Botany.80:271-279.

Barnett, H.L. and B.B. Hunter. 1998. Illustrated general of imperfect fungi (Fourth Edition). The American Phytopathological Society Press. St. Paul, MN. 218p.

Barnett, J.P. 1976. Sterilizing southern pine seeds with hydrogen peroxide. Tree Planters' Notes 27:17-19.

Berbee, J.G., F. Berbee and W.H. Brener. 1953. The prevention of damping-off of coniferous seedlings by pelleting seed. Phytopathology 43:466.

Bloomberg, W.J. 1966. The occurrence of endophytic fungi in Douglas-fir seedlings and seed. Canadian Journal of Botany 44:413-420.

Bloomberg, W.J. and J. Trelawny. 1970. Effect of thiram on germination of Douglas-fir seed. Phytopathology 60: 1111-1116.

Campbell, S.J. and T.D. Landis. 1990. Managing seedborne diseases in western forest nurseries. Tree Planters' Notes 41(4):3-7.

Carter, M.C. and L. Jones. 1962. The effect of hydrogen peroxide on the germination of loblolly and slash pine seed. USDA Forest Service, Southeastern Forest Experiment Station. Research Paper SE-141. 12p.

Cayford, J.H. and R.M. Waldon. 1967. Effects of captan on the germination of white spruce, jack and red pine seed. Forestry Chronicle 43:381-384.

Dumroese, R.K., R.L. James, D.L. Wenny, and C.J. Gilligan. 1988. Douglas-fir seed treatments: effects on seed germination and seedborne organisms. *In*: Landis, T. D. (Tech. Coord.). Proceedings: Combined Meeting of the Western Forest Nursery Associations. USDA Forest Service, Rocky Mountain Research Station, General Technical Report RM-167. pp. 155-160.

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.

Fraser, J.W. and M.J. Adams. 1980. The effect of pelleting and encapsulation on germination of some conifer seeds native to Ontario. Canadian Forestry Service, Great Lakes Forest Research Centre. Report 0-X-319. 17p.

Gordon, T.R. and R.D. Martyn. 1997. The evolutionary biology of *Fusarium oxysporum*. Annual Review of Phytopathology 35:111-128.

Hildebrand, D.M. and G.B. Dinkel. 1988. Evaluation of methyl bromide, Basamid granular, and solar heating for pre-plant pest control for fall-sown eastern red cedar at Bessey Nursery. USDA Forest Service, Rocky Mountain Region, Timber, Forest Pest, and Cooperative Forestry Management. Technical Report R2-41. 13p.

Hoff, J.A., N.B. Klopfenstein, G.I. McDonald, J.R. Tonn, M.-S. Kim, P.J. Zambino, P.F. Hessbury, J.D. Rogers,

T.L. Peever and L.M. Carris. 2004. Fungal endophytes in woody roots o Douglas-fir (*Pseudotsuga menziesii*) and ponderosa pine. (*Pinus ponderosa*). Forest Pathology 34:255-271.

James, R. L. 1986. Diseases of conifer seedlings caused by seed-borne *Fusarium* species. *In*: Shearer, R. C. (compiler). Proceedings: Conifer Tree Seed in the Inland Mountain West Symposium. USDA Forest Service, Intermountain Research Station, General Technical Report INT-203. pp. 267-271.

James, R. L. 1987. Occurrence of *Fusarium* on conifer tree seed from Northern Rocky Mountain nurseries. *In*: Landis, T. D. (tech. coord.). Proceedings: Combined Western Forest Nursery Council and Intermountain Nursery Association Meeting. USDA Forest Service, Rocky Mountain Forest and Range Experiment Station, General Technical Report RM-137. pp. 109-114.

James, R. L. 1993. *Fusarium* species associated with post-emergence damping-off and root disease of young container-grown Douglas-fir seedlings, USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Nursery Disease Notes #129. 5p.

James, R.L. 2000a. 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. 2000b. Root diseases of bareroot western larch seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Nursery Disease Notes #141. 10p

James, R.L. 2001. Effects of pre-sowing soil treatments on root colonization of 1-0 ponderosa and lodgepole pine seedlings by potentially-pathogenic fungi - USDA Forest Service Lucky Peak Nursery, Boise, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Report 01-9. 9p

James, R.L. 2002. Root disease of 1-0 bare root ponderosa pine seedlings – Lone Peak Nursery, Draper, Utah. USDA Forest Service, Northern Region, Forest Health Protection. Nursery Disease Notes No. 147. 8p.

James, R.L. 2004. Pathogenic characteristics of *Fusarium culmorum* and *Fusarium anthophilum* isolated from inland Pacific Northwest forest nurseries. USDA Forest Service, Northern Region, Forest Health Protection. Nursery Disease Notes No. 158. 12p.

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. 1989. Occurrence, characteristics, and descriptions of *Fusarium*

Stewart, J.E., M.-S. Kim, R.L. James, R.K. Dumroese and N.B. Klopfenstein. 2004. Molecular characterization of *Fusarium oxysporum* from tree nurseries: Tools for early detection of pathogens. Phytopathology 94:S99.

Stone, J.K., C.W. Bacon and J.F. White, Jr. 2000. An overview of endophytic microbes: endophytism defined. *In*: Bacon, C.W. and J.F. White, Jr. (eds.). Microbial Endophytes. Macel Dekker, Inc., New York. pp. 3-29.

Wenny, D.L. and R.K. Dumroese. 1987. Germination of conifer seeds surface sterilized with bleach. USDA Tree Planters' Notes 38(3):18-21.

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isolates from Douglas-fir seed and seedlings. USDA Forest Service, Northern Region, Forest Pest Management. Report 90-4. 23p.

James, R.L., R.K. Dumroese and D.L. Wenny. 1991. *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 and Yukon Region. Information Report BC-X-331. pp. 181-190.

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 96-6. 10p.

James, R.L., G.R. Knudsen and M.J. Morra. 2004a. Effects of preplant soil treatments on production of western white pine seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Report 04-12. 10p.

James, R.L., G.R. Knudsen and M.J. Morra. 2004b. Preplant soil treatment effects on production of Douglas-fir seedlings at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Report 04-10. 14p.

James, R. L., S. Metzger and C. J. Gilligan. 1990. Effects of soil fumigation on conifer seedling production at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Report 90-11. 18p.

James, R.L. and R. Perez. 1999. 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.

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.