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PATHOGEN INFECTION AND COLONIZATION OF CONTAINER-GROWN WHITEBARK PINE SEEDLINGS USDA FOREST SERVICE NURSERY, COEUR D'ALENE, IDAHO

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ABSTRACT

Isolations were made from 47 container-grown whitebark pine seedlings from the USDA Forest Service Nursery, Coeur d'Alene, Idaho. Sampled seedlings displayed chlorotic (dying) or necrotic (dead) foliage or appeared healthy. *Fusarium proliferatum* was isolated most frequently from all classes of seedlings. Other common *Fusarium* spp. isolated included *F. oxysporum* and *F. solani*. *Cylindrocarpon destructans* was also frequently isolated from most seedlings, but did not colonize root systems to the level of *Fusarium*. Drenching seedlings with fungicides did not stop disease development. It is likely that seedlings became infected during the first year of growth and became diseased as a result of seedling stress, conducive environmental conditions, and prolonged root colonization by pathogenic fungi. It will be difficult to grow whitebark pine seedling crops in containers without some level of disease because of high resident populations of *F. proliferatum* within greenhouses and the time requirements for adequate seedling growth under disease-conducive conditions.

INTRODUCTION

Whitebark pine (*Pinus albicaulis* Engelm.) is an important component of high elevation forest eco-systems in the Northern Region. Serious losses to this species throughout much of its natural range have recently occurred, primarily due to white pine blister rust and mountain pine beetle. As a result, there have been increased efforts to artificially regenerate many sites with whitebark pine seedlings produced in forest nurseries. Because of their slow growth,

it takes at least two years to produce seedlings large enough for outplanting. The typical growth cycle is as follows: seeds are sown in late winter or early spring of year 1 and kept in greenhouses until summer. Seedlings are then removed from greenhouses, placed outside and kept through the summer. In the fall, seedlings are returned to greenhouses and kept over winter. In the spring of year 2, a second flush of growth is artificially induced and seedlings become taller. Seedlings are normally shipped from early July through the fall of the second year. These procedures usually result in satisfactory seedling production. However, decline and mortality due to root diseases have sometimes occurred. These problems may be related to seed contamination with pathogens and prolonged exposure to nursery pathogens (James 1991, 2000a; James and Burr 2000).

Within the crop sown in early 2001 (seedlot 7425 grown in styro 91 containers), some seedlings started to decline in March of the second growing season. Seedling decline and mortality increased throughout the spring and peaked in July when greenhouse temperatures were high. Affected seedlings were generally scattered (figure 1), although some small groups of mortality were evident. Declining seedlings exhibited chlorotic foliage, often with characteristic twisting indicative of wilting in five-needle pines (figure 2)(James 1991, 2000a; James et al. 1994). By July, mortality was extensive and growers removed dead seedlings in efforts to reduce secondary disease spread (figure 3). When symptoms first became evident, applications of two fungicides (thiophanate-methyl [Cleary's 3336]; iprodione [Chipco]) were made. Fungicide applications did not reduce disease severity.

Based on previous experience with disease problems of container-grown whitebark pine seedlings at the nursery (James 1991, 2000a; James and Burr 2000) and the rapidity of symptom production and apparent disease spread, growers thought the cause of the problem may have been aggressive strains of *Fusarium* or *Cylindrocarpon*. Therefore, isolations were made from dying seedlings to test this diagnosis.

MATERIALS AND METHODS

Over the course of a few weeks, two sets of isolations were made from healthy-appearing, dying (chlorotic foliage) and dead (all foliage necrotic) seedlings. Twenty-eight seedlings were analyzed in the first set and 19 in the second. Procedures for the analysis were similar for both sets. Seedling roots were washed thoroughly to remove adhering particles of growing media. Roots were then dissected into pieces approximately 5-7 mm in length. Ten root pieces were randomly selected from each seedling; pieces were surface sterilized in a 10% bleach solution (0.525% aqueous sodium hypochlorite), rinsed in sterile, distilled water, blotted dry, and aseptically placed on a selective agar medium for *Fusarium* and closely-related fungi (Komada 1975). Plates with root pieces were incubated under diurnal cycles of cool, fluorescent light at about 24°C for at least 7 days. Emerging fungi were

Figure 1. Container-grown whitebark pine seedlings with various levels of disease symptomology - USDA Forest Service Nursery, Coeur d'Alene, Idaho. Yellow flags denote groups of seedlings dying at the same time (disease centers).

Figure 2. Dying whitebark pine seedlings with chlorotic foliage and dead seedlings with red (necrotic) foliage - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Figure 3. Diseased whitebark pine seedlings adjacent to healthy-appearing seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho. Note the empty cavities where diseased seedlings were removed.

transferred to potato dextrose agar and carnation leaf agar (Fisher et al. 1982) for fungal identification using the taxonomy of Nelson et al. (1983) and Booth (1966). Percentages of sampled seedlings infected by selected fungal genera were calculated; root colonization was determined by the percent of total root pieces colonized by particular fungi.

RESULTS

Two groups of fungi (*Fusarium* and *Cylindrocarpon*) were routinely isolated from both sets of sampled seedlings (tables 1 and 2). In the first sample, *Fusarium* spp. infected all seedlings, regardless of level of disease symptoms (table 1). However, intensity of root colonization was greater in dead seedlings compared with those that either appeared healthy or had chlorotic foliage. *Cylindrocarpon* spp. likewise infected a large proportion of seedlings, but levels of root colonization were lower than *Fusarium* (tables 1 and 2). Isolations from the second set of seedlings showed similar trends with *Fusarium* colonization somewhat higher than *Cylindrocarpon* (table 2).

Six *Fusarium* species were isolated from whitebark pine seedlings. The most common species was *F. proliferatum* (Matsushima) Nirenberg, which comprised 91% and 50% of the *Fusarium* isolates from sets 1 and 2, respectively (tables 2 and 3). Other commonly-isolated species were *F. oxysporum* Schlecht. and *F. solani*

(Mart.) Appel & Wollenw. Three other species, including *F. sambucinum* Fuckel, *F. acuminatum* Ell. & Ev., and *F. culmorum* (W.G. Smith) Sacc., were isolated at low frequencies.

Nearly all the *Cylindrocarpon* isolates obtained from whitebark pine seedlings were classified as *C. destructans* (Zins.) Scholten. (tables 1 and 2). Two isolates from healthy-appearing seedlings (set 1) were identified as *C. tenue* Bugn.

Table 1. Infection and colonization of healthy and diseased whitebark pine seedlings by *Fusarium* and *Cylindrocarpon* spp. - USDA Forest Service Nursery, Coeur d'Alene, Idaho (set 1).

Seedling Condition	Number Sampled	Infection[1]		Colonization[2]	
		Fusarium	*Cylindrocarpon*	*Fusarium*	*Cylindrocarpon*[3]
Healthy	12	100	66.7	53.3	30.8
Dying	4	100	75	57.5	22.5
Dead	12	100	91.7	91.6	26.7
All	28	100	78.6	70.4	27.9

[1] Infection based on percent of sampled seedlings that were infected by particular fungi.
[2]. Colonization based on percent of root pieces (10 sampled per seedling) colonized by particular fungi.
[3] All isolates were *C. destructans*, except two from healthy-appearing seedlings which were identified as *C. tenue*.

Table 2. Infection and colonization of diseased whitebark pine seedlings by *Fusarium* and *Cylindrocarpon* spp. - USDA Forest Service Nursery, Coeur d'Alene, Idaho (set2).

Seedling Condition	Number Sampled	Infection[1]		Colonization[2]	
		Fusarium	*Cylindrocarpon*	*Fusarium*[3]	*Cylindrocarpon*[4]
Dying	14	85.7	71.4	40.0	27.9
Dead	5	100	80.0	30.0	28.0
All	19	89.5	73.7	37.4	27.9

[1] Infection based on percent of sampled seedlings that were infected by particular fungi.
[2] Colonization based on percent of root pieces (10 sampled per seedling) colonized by particular fungi.
[3] Percent of isolates: *F. proliferatum*: 50.1; *F. oxysporum*: 31.0; *F. solani*: 11.3; *F. sambucinum*: 4.2; *F. culmorum*: 2.8.
[4] All isolates were *C. destructans*.

Table 3. *Fusarium* species isolated from healthy and diseased whitebark pine seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho (set 1)[1].

Seedling Condition	*Fusarium* Species[2]					
	FPRO	FOXY	FSOL	FSAM	FACU	ALL
Healthy	62	0	0	0	2	64
Dying	19	0	4	0	0	23
Dead	101	3	7	1	1	113
All (Total)	182	3	11	1	3	200
Percent	91.0	1.5	5.5	0.5	1.5	100

[1] Values in table are number of isolates of particular species.
[2] FPRO = *F. proliferatum*; FOXY = *F. oxysporum*; FSOL = *F. solani*; FSAM = *F. sambucinum*: FACU = *F. acuminatum*.

DISCUSSION

The need for whitebark pine seedlings to regenerate high elevation sites increases each year in the Northern Region. Seedlings must be produced in containers within greenhouses to ensure they are of sufficient size to outplant. Even so, it takes nearly two full growing seasons in the nursery to produce large enough stock. This is in contrast with most other container conifer species at the Coeur d'Alene Nursery which are produced in about 6 months.

Conditions under which seedlings are grown in greenhouses are often ideal for diseases (James 1984, 1988; James et al. 1988a). High humidity and conducive temperatures provide ample opportunities for pathogenic fungi to infect and colonize seedlings (James et al. 1988a, 1990). As a result, many seedlings tend to become infected when they are very young (James et al. 1987, 1991) They may or may not display disease symptoms as they age (James et al. 1987, 1988a, 1991). Pathogen inoculum comes from infested seed (Dumroese et al. 1988; James 1986, 1987a, 1987b), reused containers (James et al. 1988d; James and Woolen 1989), and organic matter within or near greenhouses (James 1984; James et al. 1991). It is probably impossible to completely eliminate pathogen inoculum. Therefore, some level of seedling infection is inevitable. However, disease severity may vary widely and can be affected by cultural practices. For example, using high-quality, pathogen-free seed is important in reducing disease severity (James 1986, 1987b; James et al. 1988b, 1988c). Whitebark pine seed is often extensively colonized with many different fungi, including those capable of eliciting disease (James 2000a; James and Burr 2000). Seed contamination can increase during individual seed clipping, which is required for stimulate germination (James and Burr 2000). Reducing level of seed colonization by potential pathogens prior to stratification will improve chances for disease-free seedlings after sowing (James 1986, 1987b). Running water rinses (for at least 48 hours) should be mandatory (James 1987a); chemical treatments with either bleach (sodium hypochlorite), hydrogen peroxide, or fungicides may be necessary to further reduce fungal contamination (James 1986, 1987b). Another approach is to treat severely-

contaminated seeds with hot water (James et al. 1988c). Water temperatures must be sufficient to kill seedcoat fungi, but not high enough to adversely affect germination. Adequate sanitation of reused containers (James et al. 1988d, 1990) and the interior of greenhouses (James 1984; James et al. 1990) should also reduce disease severity.

A major problem with growing container whitebark pine seedlings in nurseries is the long production cycle required. During the two-year cycle, seedlings undergo several periods of active growth, followed by dormancy. They are also usually exposed to extremes in environmental conditions, i.e., from high to low temperatures and wet to dry periods. Because of these extremes, they are undoubtedly stressed to some extent. If potential pathogens are present on roots early in the growth cycle, when seedlings become stressed, pathogen activity increases and disease often results (James et al. 1987, 1988a). Therefore, during the two-year production cycle, pathogen activity within roots probably increases and decreases several times. If root colonization is extensive and probably beyond threshold levels and fungal populations are sufficiently aggressive, disease results. There is probably some "point of no return" after which host resistance breaks down and pathogens progress unabated and cause disease symptoms and ultimately seedling death. If infected seedlings can remain non-diseased, i.e., lack disease symptoms, they can be expected perform well once outplanted on forest sites (Dumroese et al. 1993, 2000), This occurs primarily because pathogens obtained in nurseries are replaced by other, non-pathogenic mycoflora following out-planting.

Trying to control root disease by applications of chemical fungicides after the onset of symptoms is often ineffective (Dumroese et al. 1990; James et al. 1990). Level of root colonization and location of pathogens, often deep within plugs (James 1998), makes it difficult for toxic chemicals to reach their targets at sufficient concentrations to adversely affect pathogens (Dumroese et al. 1990; James et al. 1990). Therefore, it is important to maintain high seedling vigor throughout production as well as limiting host exposure to pathogen inoculum. This may be especially difficult to do for whitebark pine seedlings.

These problems are exacerbated by the fact that the major fungus isolated from both healthy-appearing and diseased seedlings was *F. proliferatum*. This species is commonly associated with root-diseased conifer seedlings at the Coeur d'Alene Nursery (James 1990; James and Perez 1998) and pathogenicity tests have confirmed that it is usually quite virulent on conifer seedlings (James 1997; James et al. 1991, 1995, 1997). This is in contrast to several other *Fusarium* and *Cylindrocarpon* spp., which were also isolated from diseased whitebark pine seedlings (James et al. 1994). These other fungi are usually not as virulent as *F. proliferatum* (James 2000b; James and Perez 2000; James et al. 2000). Therefore, severe whitebark pine seedling disease may be related to presence of highly virulent pathogen strains coupled with conducive disease conditions. This combination resulted in higher than normal disease levels, which adversely affected production goals. Fortunately, subsequent seedling crops

were not so severely diseased. Vigilance is required by growers to prevent severe disease in the future by initiating sanitation efforts on seeds, containers, and the growing environ-ment. Prevention is the best way to assure that future seedling crops do not become severely diseased.

LITERATURE CITED

Booth, C. 1966. The genus *Cylindrocarpon*. Commonwealth Mycological Institute. Kew, Surrey, England. Mycological Papers No. 104. 56p.

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.

Dumroese, R.K., R.L. James and D L. Wenny. 1990. Trial of a granular etridiazole and thiophanate-methyl mixture to control *Fusarium* root disease of container-grown Douglas-fir seedlings. New Forests 4:231-236.

Dumroese, R.K., R.L. James and D L. Wenny. 1993. *Fusarium* root infection of container-grown Douglas-fir: effect on survival and growth of outplanted seedlings and persistence of the pathogen. New Forests 7:143-149.

Dumroese, R.K., R.L. James and D.L. Wenny. 2000. An assessment of *Cylindrocarpon* on container western white pine seedlings after outplanting. Western Journal of Applied Forestry 15(1):5-7.

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.

James, R.L. 1984. Diseases of containerized conifer seedlings. *In*: Dubreuil, S. H. (compiler). Proceedings of the 31st Western International Forest Disease Work Conference, Coeur d'Alene, Idaho. pp. 17-23.

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. 1987a. Effects of water rinse treatments on occurrence of fungi on spruce seed from the Towner Nursery, North Dakota. USDA Forest Service, Northern Region, Forest Pest Management. Report 87-5. 4p.

James, R.L. 1987b. 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 Inter-mountain Nursery Association Meeting. USDA Forest Service, Rocky Mountain Forest and Range Experiment Station, General Technical Report RM-137. pp. 109-114.

James, R.L. 1988. Diseases of conifer seedlings associated with *Cylindrocarpon* species: a review. USDA Forest Service, Northern Region, Forest Pest Management. Nursery Disease Notes No. 76. 14p.

James, R.L. 1990. Container-grown lodgepole pine seedling disease - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Nursery Disease Notes No. 100. 6p.

James, R.L. 1991. Cylindrocarpon root disease of container-grown whitebark pine seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Report 91-8. 10p.

James, R.L. 1997. A short review of *Fusarium* section *Liseola*: implications for conifer seedling production. *In*: James, R. L. (editor). 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. 34-41.

James, R.L. 1998. Quantification of conifer seedling root colonization by *Fusarium* and *Cylindrocarpon* species. USDA Forest Service, Northern Region, Forest Health Protection. Nursery Disease Notes No. 135. 8p.

James, R.L. 2000a. Diseases associated with whitebark pine seedling production – USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Report 00-8. 11p.

James, R.L. 2000b. 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. and K.E. Burr. 2000. Diseases associated with whitebark pine seedling production - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Report 00-8. 11p.

James, R.L., R.K. Dumroese and D.L. Wenny. 1988a. *Fusarium* diseases of containerized conifer seedlings in northern Rocky Mountain nurseries: infection, symptom production and pathogenicity of associated fusaria. Phytopathology 78(12):1533.

James, R.L., R.K. Dumroese and D.L. Wenny. 1988b. *Fusarium* diseases of containerized conifer seedlings in northern Rocky Mountain nurseries: sources of inoculum and control tests. Phytopathology 78(12):1607.

James, R.L., R.K. Dumroese and D.L. Wenny. 1988d. Occurrence and persistence of *Fusarium* within styro-block and Ray Leach containers. *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. 145-148.

James, R.L. , R.K. Dumroese and D L. Wenny. 1990. Approaches to integrated pest management of Fusarium root disease in container-grown conifer seedlings. *In*: Rose, R., S. J. Campbell and T. D. Landis (eds.). Target Seedling Symposium: Proceedings, Combined Meeting of the Western Forest Nursery Associations. USDA Forest Service, Rocky Mountain Research Station, General Technical Report RM-200. pp. 240-246.

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., R.K. Dumroese and D.L. Wenny. 1994. Observations on the association of *Cylindrocarpon* spp. with diseases of container-grown conifer seedlings in the inland Pacific Northwest of 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., R.K. Dumroese and D.L. Wenny. 1995. *Fusarium proliferatum* is a common, aggressive pathogen of container-grown conifer seedlings. Phytopathology 85(10): 1129.

James, R.L., R.K. Dumroese and D.L. Wenny. 1997. Pathogenicity of *Fusarium proliferatum* in container-grown Douglas-fir seedlings. *In:* James, R. L. (editor). 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. 26-33.

James, R.L., R.K. Dumroese, D.L. Wenny, J.F. Myers and C.J. Gilligan. 1987. Epidemiology of *Fusarium* on containerized Douglas-fir seedlings. I. Seed and seedling infection, symptom production, and disease progression. USDA Forest Service, Northern Region, Forest Pest Management. Report 87-13. 22p.

James, R.L., C.J. Gilligan, R.K. Dumroese, and D.L. Wenny. 1988c. Microwave treatments to eradicate seedborne fungi on Douglas-fir seed. USDA Forest Service, Northern Region, Forest Pest Management. Report 88-7. 8p.

James, R.L. and R. Perez. 1998. *Fusarium proliferatum* root disease on container-grown Douglas-fir seedlings from Pacific Northwest Region seedlots - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Nursery Disease Notes No. 138. 6p.

James, R.L. and R. Perez. 2000. Pathogenic characteristics of *Fusarium solani* isolated from inland Pacific Northwest forest nurseries. USDA Forest Service, Northern Region, Forest Health Protection. Report 00-15. 12p.

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.

James, R.L. and R.L. Woollen. 1989. An evaluation of the efficacy of hot water-chemical treatments to clean styroblock containers - Champion Timberlands Nursery, Plains, Montana. USDA Forest Service, Northern Region, Forest Pest Management. Report 89-5. 8p.

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.

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