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DISEASES ASSOCIATED WITH WHITEBARK PINE SEEDLING PRODUCTION USDA FOREST SERVICE NURSERY, COEUR D'ALENE, IDAHO

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ABSTRACT

Whitebark pine is an important reforestation species in the northern Rocky Mountains for enhancement of wildlife habitat. Production of container-grown whitebark pine seedlings at the USDA Forest Service Nursery in Coeur d'Alene, Idaho has been increasing the past several years. Diseases continue to be an important limiting factor in seedling production. Recent evaluations implicated *Fusarium solani* and *F. proliferatum* as important pathogens of young germinants and seedlings. Both fungal species commonly contaminated whitebark pine seed prior to sowing. Pathogen spread during stratification resulted in very high levels of seed contamination. *Fusarium* spp. adversely affected seed germination as well as initiated high seedling disease levels. Not all seedlots were equally affected by diseases, even though most seeds were contaminated. Seedling vigor, probably related to seed germinative energy, may have been an important factor affecting disease severity. Efforts to reduce contamination by mechanical brushing or treatments with hydrogen peroxide or bleach solutions were ineffective. Seed-borne inoculum is important in disease epidemiology. Improved techniques to reduce level of pathogen contamination and spread during seed processing are required.

INTRODUCTION

Whitebark pine (*Pinus albicaulis* Englem.) seedling production at the USDA Forest Service Nursery in Coeur d'Alene, Idaho has steadily increased over the past several years. The production goal for 1998 was for 250,000 seedlings, most of which were distined for wildlife habitat restoration on federal lands in the Northern and Intermountain Regions. Diseases of container-grown whitebark pine seedlings caused primarily by *Cylindrocarpon destructans* (Zins.) Scholten. were previously shown to be important (James 1991a). This fungus attacked seedling stems and roots, causing damping-off on young germinants and root decay on older seedlings. Although *Fusarium* spp. were often isolated from whitebark pine seeds, they were not considered as important as *C. destructans* in causing seedling diseases (James 1991a).

Growers at the Coeur d'Alene Nursery recently found higher than normal levels of disease in young container-grown whitebark pine seedlings. Diseased seedlings displayed post-emergence damping-off (Hartley and others 1918; Vaartaja and Cram 1956) with noticeable purple-black stem lesions. Lesions made seedling stems very flexible and easily bent

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over. Diseases seedlings were scattered throughout most seedlots, although lots 78 and 95 from the Gallatin National Forest were most severely affected.

During germination tests, workers found excessive mold on seeds which might adversely affect germination. Several seed assays were conducted to identify and quantify fungi and to evaluate ways of reducing fungal contamination. Isolations were also made from diseased seedlings to determine associated fungi.

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MATERIALS AND METHODS

Five whitebark pine seedlots were evaluated: B090 (Sawtooth National Forest-designated B), J090 (Sawtooth National Forest-designated J), 6783 (Gallatin National Forest-designated 78), 6953 (Gallatin National Forest-designated 95), and 7029 (Targhee National Forest-designated 7029). Twenty-five seeds were randomly collected from frozen storage barrels for each seedlot. Seeds were aseptically placed on a selective agar medium for *Fusarium* spp. and closely related fungi (Komada 1975). Plates with seeds were incubated under diurnal cycles of cool, fluorescent light at about 24°C for 7-10 days, after which fungi growing from seeds were identified. Percent seed colonized by particular fungi was determined.

Prior to sowing, whitebark pine seeds are normally rinsed in running tap water for 48 hours. They are then warm stratified at 21-22°C for 30 days followed by a 60 day cold (1-2°C) stratification. Seeds are typically rinsed in running tap water for about 1 hour each week during both stratification periods. Following stratification, seedcoats are normally cut, exposing female gametophyte tissues, to improve germination.

A second seed assay was conducted during the cold stratification. Nineteen randomly collected seeds from three different bags were selected for assay of associated fungi. Seeds were aseptically placed directly on the selective agar medium and incubated as described above. Selected *Fusarium* isolates were transferred to carnation leaf (Fisher and others 1982) and potato dextrose agar for identification using the taxonomy of Nelson and others (1983).

The third seed assay was conducted on 23 randomly selected seeds located within germination trays which had been incubated for 21 days at 22°C. All assayed seeds had abundant external fungal growth. Selected seeds were carefully lifted with sterilized tweezers and placed directly on the selective agar medium and incubated as described above. Selected *Fusarium* isolates were identified.

Attempts were made to reduce surface mold contamination by subjecting seeds to three different treatments prior to stratification: (1) brushing to mechanically remove surface seedcoat fungi, (2) immersing seeds in a 30% hydrogen peroxide (H_2O_2) solution for 30 minutes followed by water rinsing, and (3) immersing seeds in an aqueous solution of sodium hypochlorite (two parts commercial bleach and 3 parts tap water for 10 min. followed by a water rinse)(Wenny and Dumroese 1987). In some cases, seeds were exposed to two of the treatments, e.g., brushing followed by H_2O_2 treatment. Following treatment, 50 post-stratification seeds were aseptically placed on the selective agar medium and incubated as described above. Selected *Fusarium* isolates were identified; percent of treated seeds colonized by different fungi was calculated.

Fifteen 2- to 3-week-old seedlings with typical post-emergence damping-off symptoms were collected from the greenhouse and analyzed in the laboratory. Seedlings were carefully removed from containers and their roots and stems washed thoroughly. They were then surface sterilized in 0.525% aqueous sodium

hypochlorite (10% bleach), rinsed in sterile water, blotted dry, and dissected into three portions: stems with apparent lesions, roots, and the cotyledons. Tissue pieces were aseptically placed on the selective medium, incubated as described previously, with selective *Fusarium* isolates processed for identification. Percent colonization of the three tissue types by selected fungi was determined. Thirty-nine additional diseased seedlings were collected at about 8, 12, and 14 weeks after sowing. Samples were processed as described above with the exception that only roots were assayed. Percent of seedlings infected and root colonization rates (James 1998) were calculated.

The problem of determining when and how seeds became contaminated with *Fusarium* prompted sampling germination chambers and stratification rooms for *Fusarium* spores. Six 9-cm petri plates with selective agar medium (Komada 1975) were exposed for 15 minutes in each of two germination chambers; 12 similar plates were exposed for 2 hours in each of two stratification rooms. After exposure, plates were incubated as described above and fungi arising from airborne spores identified. Selected pieces of organic debris were collected from floors of both stratification rooms and placed directly on the selective medium; plates were incubated as before and associated *Fusarium* spp. identified.

RESULTS

Whitebark pine seeds from frozen storage were colonized by four major groups of fungi (table 1). The most common fungi were *Penicillium* spp.; other genera on seeds included *Aspergillus*, *Trichoderma*, and *Botrytis*. All these fungi may be saprophytes which readily colonize conifer seeds (Anderson 1986; James 1999; James and Genz 1981, 1982). *Botrytis* may also be an important pathogen of conifer seedlings, especially those within greenhouses (James 1984a; James and others 1983).

Seeds sampled from cold stratification bags and germination trays had very high levels of *Fusarium* contamination (table 2). *Fusarium solani* (Mart.) Appel & Wollenw. was isolated from all sampled seed during stratification and was also common on germinating seed *Fusarium proliferatum* (Matsushima) Nirenberg was common on germinating seeds.

Treatments evaluated to reduce *Fusarium* seed contamination were unsatisfactory (table 3). Neither mechanical brushing nor exposure to hydrogen peroxide or bleach solutions were effective. *Fusarium solani* was especially persistent on seeds from four of the five seedlots (J, 78, 95, and 7029) regardless of treatment.

Seedlot B was extensively contaminated with *F. proliferatum*. Treatments with surface sterilants also did not always eliminate common saprophytic seed colonizers such as *Trichoderma* and *Penicillium* spp.

Both *F. solani* and *F. proliferatum* were often isolated from whitebark pine germinants with post-emergence damping-off symptoms (table 4). *Fusarium solani* was isolated from stem lesions, roots, and cotyledons of all damped-off seedlings sampled. *Fusarium proliferatum* was isolated less frequently, but commonly colonized stem lesions and roots of diseased germinants. Another *Fusarium* species, *F. scirpi* Lambotte & Fautr., was isolated from roots of one diseased germinant. *Cylindrocarpon destructans* was also isolated from stem lesions and roots of a few diseased plants, but was not as commonly associated with diseased whitebark pine seedlings as previously (James 1991a).

Isolations from diseased seedlings throughout the growth cycle yielded high levels of *Fusarium* spp. (table 5). Both *F. solani* and *F. proliferatum* continued to colonize seedling roots as seedlings became older. Another *Fusarium* species, *F. sambucinum* Fuckel, was isolated once from seedling roots. Root systems were also extensively colonized with saprophytic *Trichoderma* and *Penicillium* species during all sampling periods.

Airborne *Fusarium* spores were not collected in either germination chambers or stratification rooms (table 6); the only fungi recovered from the air were considered non-pathogens. However, *F. solani* and *F. equiseti* (Corda) Sacc. were isolated at low levels from organic debris on the floor of one stratification room.

Table 1. Fungal colonization of whitebark pine seed from frozen storage USDA Forest Service Nursery, Coeur d' Alene, Idaho.

	Percent Seed Colonization[1]			
Seedlot[2]	*Penicillium*	*Aspergillus*	*Trichoderma*	*Botrytis*
B	48	40	12	0
J	96	4	0	0
78	60	0	16	24
95	96	4	0	0
7029	100	0	0	0
Average	80.0	9.6	3.4	4.8

[1]Twenty-five randomly collected seeds sampled per seedlot.

[2]Seedlots: B & J = Sawtooth National Forest; 78 & 95 = Gallatin National Forest; 7020 = Targhee National Forest.

Table 2. Occurrence of *Fusarium* spp. on whitebark pine seed sampled from cold stratification bags and germination trays at the USDA Forest Service Nursery, Coeur d'Alene, Idaho[1].

Sample Location[2]	*Fusarium solani*	*Fusarium proliferatum*
Strat. Bag #1	100	0
Strat. Bag #2	100	0
Strat. Bag #3	100	0
Average	100	0
Germ. Tray #1	67	50
Germ. Tray #2	63	63
Germ. Tray #3	78	22
Average	69.3	45.0

[1]Numbers in table are percent of sampled seed colonized by particular *Fusarium* spp.

[2]First three samples from bags of seeds undergoing cold stratification; second three samples from standard germination trays within incubator.

Table 3. Effects of mechanical brushing, hydrogen peroxide and sodium hypochlorite treatment on colonization of whitebark pine seed by selected fungi at the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Percent Seed Colonization				
Treatment[1]	*Fusarium solani*	*F. proliferatum*	*Trichoderma*	*Penicillium*
Lot B; Bl	0	100	78	20
Lot B; Br - Bl	0	100	36	0
Lot J; Bl	100	0	36	18
Lot J; Br - Bl	100	0	36	16
Lot 78; H_2O_2	100	6	8	54
Lot 78; Br - H_2O_2	100	0	0	0
Lot 95; H_2O_2	100	0	26	0
Lot 95; Br - H_2O_2	100	0	58	0
Lot 7029; Bl	100	0	22	38
Average	77.8	22.9	33.3	14.2

[1]Treatments: Bl = aqueous sodium hypochlorite (bleach) at 2 parts bleach, 3 parts tap water for 10 minutes followed by tap water rinse; Br = mechanical brushing; H_2O_2 = 30% hydrogen peroxide for 30 minutes followed by tap water rinse. Seedlots B & J = Sawtooth National Forest; 78-95 = Gallatin National Forest; 7020 = Targhee National Forest.

Table 4. Association of selected fungi with post-emergence damping-off of young whitebark pine germinants at the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Percent Germinant Infection and Colonization[1]						
Location[2]	FSOL	FPRO	FSCI	CYDE	TRI	PEN
Les Inf	100	6.7	0	6.7	0	0
Les Colon	89.4	4.5	0	6.1	0	0
Root Inf	100	20	6.7	20	60	26.7
Root Colon	45	5.5	1	5.5	38.5	11
Cot Inf	100	0	0	0	0	0
Cot Colon	90.5	0	0	0	0	0
All Inf	100	26.7	6.7	20	60	26.7
All Colon	66.8	4.5	0.6	5.1	19.7	5.6

[1]Percent of germinants (infection) and sampled pieces (colonization) with particular fungus; FSOL = *Fusarium solani*; FPRO = *Fusarium proliferatum*; FSCI = *Fusarium scirpi*; CYDE = *Cylindrocarpon destructans;* TRI = *Trichoderma* spp.; PEN = *Penicillium* spp.

[2]Isolation location: Les = Lesions on main stem; Root = root system; Cot = base of cotyledons where attached to main stem; Inf = percent of seedlings infected (15 sampled); Colon = percent of sampled tissue pieces colonized.

Table 5. Colonizaiton of diseased whitebark pine seedling roots with selected fungi at the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Percent Seedling Infection and Colonization[1]					
Sample Time[2]	FSOL	FPRO	FSAM	TRI	PEN
8 wks - Inf	100	100	0	100	100
8 wks - Colon	47.4	52.6	0	52.6	84.2
12 wks - Inf	63	37	11.1	48.1	11.1
12 wks - Colon	43.2	25.6	6.4	22.4	3.2
14 wks - Inf	60	40	0	90	40
14 wks - Colon	40	21.1	0	38.9	12.2

[1]Percent of seedlings (infection) and sampled root pieces (colonization) with particular fungus: FSOL = *Fusarium solani;* FPRO = *Fusarium proliferatum;* FSAM = *Fusarium sambucinum*; TRI = *Trichoderma* spp.; PEN = *Penicillium* spp.

[2]Eight,12, and 14 week samples consisted of 2, 27, and 10 seedlings, respectively; Inf = percent of seedlings infected; Colon = percent of sampled root pieces colonized.

Table 6. Fungal spore distribution within germination chambers and stratification rooms at the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Percent Exposed Spore Traps Colonized[1]				
Location[2]	*Penicillium*	*Aspergillus*	Unidentified Fungi	None[3]
Germ A	33.3	0	0	66.7
Germ B	0	16.7	0	83.3
Strat A	8.3	0	91.7	0
Strat B	41.7	0	33.3	41.7

[1]Spore traps consisted of 9 cm petri plates with Komada's medium; 6 traps were exposed for 15 minutes in each germination chamber; 12 traps were exposed for 2 hrs. in each stratification room; values in table are percent of exposed spore traps infected with particular fungi.

[2]Germ = germination chambers; Strat = stratification rooms.

[3]No fungi found on spore traps after exposure period.

DISCUSSION

Fusarium solani and *F. proliferatum* were the most important pathogens colonizing seed and causing disease of young whitebark pine germinants and older seedlings at the nursery. *Fusarium solani* frequently colonizes conifer seeds, including Douglas-fir (James 1983; James 1984b, 1987d), ponderosa pine (James and Genz 1981, 1982) and several other pine species (Anderson 1986; James 1987d). This fungus also causes damping-off and root disease of conifer seedlings (Hartley and others 1918; Hocking 1968; Matuo and Chiba 1966; Vaartaja 1967; Vaartaja and Bumbieris 1967; Vaartaja and Cram 1956); certain isolates may be very virulent on pine seedlings (Hildebrand 1985; Hocking and others 1968). However, *F. solani* is best known asa root pathogen of agricultural crops, especially beans (Guerra and Anderson 1985; Hall and Phillips 1992; Maurer and Baker 1965) and peas (Hadwiger and Beckman 1980; Hadwiger and others 1981; Kendra and Hadwiger 1987). Other important agricultural hosts include alfalfa (Graham and others 1979), various cereals (Gordon and Sprague 1941; Sturz and Bernier 1991), potatoes (Olsson 1989), and cucurbits (Samac and Leong 1989; Schippers and Old 1974). *Fusarium solani* is a common soil inhabitant (Joffe and Palti 1977; Kommedahl and others 1975; Summerell and others 1993), where it resides primarily as resting chlamydospores (El-Ani 1988; Schippers and Old 1974). When environmental conditions are conducive and a suitable host is nearby (Griffin 1970; Schroth and Snyder 1961), spores germinate and hyphae readily colonize roots. *Fusarium solani* can quickly produce voluminous numbers of spores under conducive environmental conditions (Cappellini and Peterson 1965) which are disseminated in water (Gregory and others 1959; Snyder 1981) and, to a lesser extent, in air (Burgess 1981). Since the fungus is metabolically active at low temperatures (Cook and Flentje 1965; Schuerger and Mitchell 1992), cool stratification conditions are conducive for spread of *F. solani*.

Fusarium proliferatum is frequently associated with container-grown seedling root diseases (James 1987a, 1987b, 1987c, 1991b, 1993) and may be an aggressive pathogen of conifer seedlings (Huang and Kuhlman 1990; James and others 1995; Shrimptom and Williams 1989; Viljoen and others 1995). This fungal species is infrequently isolated from conifer seeds (Barrows-Broaddus 1990; Fraedrich and Miller 1995; James 1999). Because *F. proliferatum* produces extensive chains of microconidia which are air and water dispersed (Fisher and others 1983; Nelson and others 1990), if only a few seeds are initially infected, the pathogen can rapidly spread throughout a seedlot during stratification (Kliejunas 1985; Sutherland and van Eerden 1980).

Many *Fusarium* spores produce a mucilage coating allowing tight adherence to seedcoat surfaces (Schuerger and Mitchell 1993). *Fusarium* spp. may also readily penetrate conifer seedcoats and colonize internal tissues (James and Genz 1982). As a result, surface sterilants or mechanical brushing may not eliminate *Fusarium* on seed (Edwards and Sutherland 1979; Fuller and Hildebrand 1985) which was confirmed in this evaluation.

Whitebark pine seeds are notoriously difficult to germinate in nurseries because of inherent dormancy. To enhance germination in nurseries, mechanical cutting of the seedcoat is required. Fungal contamination likely spreads during this process (James 1991a) resulting in most seeds becoming infected from only a few initially contaminated seeds. Seed infection with pathogens capable of eliciting disease on young seedlings should be reduced whenever possible.

Nursery personnel found that the best performing whitebark pine seedlot was 7029 (Targhee National Forest), while the poorest seedlots were 78 and 95 (Gallatin National Forest). Although diseased seedlings were found in each seedlot, seed germination was poorer and diseases more common in seedlots 78 and 95. However, potentially pathogenic *Fusarium* spp. were very common on all seedlots. Therefore, seedling vigor, as manifested by seed germination capacity and energy (Larson 1963), was probably an important factor limiting disease under conditions of high pathogen inoculum potential. Apparently, vigorous seedlings are able to overcome *Fusarium*-associated diseases. Therefore, it is important that only high-quality seed be used to grow whitebark pine seedlings.

LITERATURE CITED

Anderson, R.L. 1986. Checklist of micro-organisms associated with tree seeds in the world, 1985. USDA Forest Service, Southeastern Forest Experiment Station, General Technical Report SE-39. 34p.

Barrows-Broaddus, J. 1990. Colonization of cones and seed of loblolly pine following inoculation with *Fusarium subglutinans*. Plant Disease 74:1002-1005.

Burgess, L.W. 1981. General ecology of the Fusaria. In: Nelson, P.E., T.A. Toussoun and R.J. Cook (eds.). *Fusarium*: Diseases, Biology & Taxonomy. The Pennsylvania State University Press, University Park. pp. 225-235.

Cappellini, R.A. and J.L. Peterson. 1965. Macroconidium formation in submerged cultures by a non-sporulating strain of *Gibberella zeae*. Mycologia 57:962-966.

Cook, R.J. and N.T. Flentje. 1965. Influence of soil water on growth and survival of *Fusarium solani* f.sp. *pisi* in the rhizosphere of pea. Phytopathology 55:1051-1085.

Edwards, D.G.W. and J.R. Sutherland. 1979. Hydrogen peroxide treatment of *Abies* seeds. Canadian Forestry Service, BiMonthly Research Notes 35:3-4.

El-Ani, A.S. 1988. The chlamydospore in *Fusarium solani*. Mycologia 80:885-887.

Fisher, N.L., L.W. Burgess and T.A. Toussoun. 1983. *Taxonomic* importance of microconidial chains in *Fusarium* section *Liseola* and effects of water potential on their formation. Mycologia 75:693-698.

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.

Fraedrich, S.W. and T. Miller. 1995. Mycoflora associated with slash-pine seeds from cones collected at seed orchards and cone-processing facilities in the south-eastern USA. European Journal of Forest Pathology 25:73-82.

Fuller, L.R. and D.M. Hildebrand. 1985. Effects of cold stratification and hydrogen peroxide treatments on seeds of three Rocky Mountain conifer species. USDA Forest Service, Rocky Mountain Region, Timber, Forest Pest and Cooperative Forestry Management. Technical Report R2-32. 20p.

Gordon, W.L. and R. Sprague. 1941. Species of Fusarium associated with root rots of the Gramineae in the northern Great Plains. Plant Disease Reporter 25:168-180.

Graham, J.H., D.L. Stuteville, F.I. Frosheiser and D.C. Erwin. 1979. Compendium of alfalfa diseases. The American Phytopathological Society, St. Paul, MN. 65p.

Gregory, P.H., E.J. Guthrie and M.E. Bunce. 1959. Experiments of splash dispersal of fungus spores. Journal of General Microbiology 20:328-354.

Griffin, G.J. 1970. Carbon and nitrogen requirements for macroconidial germination of *Fusarium solani*: dependence on conidial density. Canadian Journal of Microbiology 16:733-740.

Guerra, D. and A.J. Anderson. 1985. The effect of iron and boron amendments on infection of bean by *Fusarium solani*. Phytopathology 75:992-995.

Hadwiger, L.A. and J.M. Beckman. 1980. Chitosan as a component of pea-*Fusarium solani* interactions. Plant Physiology 66:205-211.

Hadwiger, L.A., J.M. Beckman and M.J. Adams. 1981. Localization of fungal components in the pea-*Fusarium* interaction detected immunochemically with anti-chitosan and anti-fungal cell wall antisera. Plant Physiology 67:170-175.

Hall, R. and L.G. Phillips. 1992. Effects of crop sequence and rainfall on population dynamics of *Fusarium solani* f.sp. *phaseoli* in soil. Canadian Journal of Botany 70:2005-2008.

Hartley, C., T.C. Merrill and A.S. Rhoades. 1918. Seedling diseases of conifers. Journal of Agricultural Research 15:521-558.

Hildebrand, D.M. 1985. Pathogenicity of *Fusarium* spp. to lodgepole pine. USDA Forest Service, Rocky Mountain Region, Forest Pest Management. Biological Evaluation R2-85-8. 6p.

Hocking, D. 1968. Fungi associated with damped-off and healthy pine seeds and with seed in East African pine nurseries. Transactions of the British Mycological Society 51:221-226.

Hocking, D., E.C. Setlift and A.A. Jaffer. 1968. Potential pathogenicities of fungi associated with damped-off pine seedlings in East African pine nurseries. Transactions of the British Mycological Society 51:227-232.

Huang, J.W. and E.G. Kuhlman. 1990. Fungi associated with damping-off of slash pine seedlings in Georgia. Plant Disease 74:27-30.

James, R.L. 1983. Occurrence of *Fusarium* on Douglas-fir seed from the Coeur d'Alene Nursery. USDA Forest Service, Northern Region, Forest Pest Management. Nursery Disease Notes No. 4. 11p.

James, R.L. 1984a. Biology and management of Botrytis blight. *In:* Murphy, P.M. (compiler). The Challenge of Producing Native Plants for the Intermountain Area: Proceedings: Intermountain Nurseryman's Association 1983 Conference. USDA Forest Service, General Technical Report INT-168. pp. 39-43.

James, R.L. 1984b. Fungi colonizing Douglas-fir seed at the Champion Timberlands Nursery, Plains, Montana. USDA Forest Service, Northern Region, Forest Pest Management. Report 84-13. 3p.

James, R.L. 1987a. Containerized lodgepole pine needle tip necrosis - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Nursery Disease Notes No. 63. 3p.

James, R.L. 1987b. Containerized western larch seedling mortality - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Report 87-1. 7p.

James, R.L. 1987c. Containerized western white pine seedling root disease - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Nursery Disease Notes No. 66. 4p.

James, R.L. 1987d. Occurrence of *Fusarium* spp. on conifer seed from Northern Rocky Mountain nurseries *In*: Landis, T.D. (tech. coord.). Proceedings: Combined Western Nursery Council and Intermountain Nursery Association Meeting. USDA Forest Service, General Technical Report RM-137. pp. 104-114.

James, R.L. 1991a. 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. 1991b. Wilting of container-grown western white pine seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Nursery Disease Notes No. 123. 5p.

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 No. 129. 5p.

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. 1999. Ponderosa pine seed fungal contamination: effects of stratification and sterilizing treatments. USDA Forest Service, Northern Region, Forest Health Protection. Report 99-3. 11p.

James, R.L., R.K. Dumroese and D.L. Wenny. 1995. *Fusarium proliferatum* is a common, aggressive pathogen of container-grown conifer seedlings. Phytopathology 85:1129.

James, R.L. and D. Genz. 1981. Ponderosa pine seed treatments: effects on seed germination and disease incidence. USDA Forest Service, Northern Region, Forest Pest Management. Report 81-16. 13p.

James, R.L. and D. Genz. 1982. Evaluation of fungal populations on ponderosa pine seed. USDA Forest Service, Northern Region, Forest Pest Management. Report 82-22. 21p.

James, R.L., J.Y. Woo and J.F. Myers. 1983. Evaluation of fungicides to control Botrytis blight of containerized western larch and lodgepole pine seedlings at the Coeur d'Alene Nursery, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Report 82-17. 13p.

Joffe, A.Z. and J. Paltri. 1977. Species of *Fusarium* found in uncultivated desert type soils in Israel. Phytoparasitica 5:119-121.

Kendra, D.K. and L.A. Hadwiger. 1987. Cell *death* and membrane leakage not associated with the induction of disease resistance in peas by chitosan or *Fusarium solani* f.sp. *phaseoli.* Phytopathology 77:100-106.

Kliejunas, J. 1985. Effect of selected chemicals on mold development during conifer seed stratification and on subsequent germination. USDA Forest Service, Pacific Southwest Region, Forest Pest Management. Report 85-35. 7p.

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.

Kommedahl, T., C.S. Windels and D.S. Lang. 1975. Comparisons of *Fusarium* populations in grasslands of Minnesota and Iceland. Mycologia 67:38-44.

Larson, M.M. 1963. Initial root development of ponderosa pine seedlings as related to germination date and size of seed. Forest Science 9:456-460.

Matuo, T. and O. Chiba. 1966. Species and formae speciales of *Fusarium* causing damping-off and root rot of coniferous seedlings in Japan. Annals of the Phytopathological Society of Japan 32:14-22.

Maurer, C.L. and R. Baker. 1965. Ecology of plant pathogens in soil. I. Influence of chitin and lignin amendments on development of bean root rot. Phytopathology 54:1425-1426.

Nelson, P.E., L.W. Burgess and B.A. Summerell. 1990. Some morphological and physiological characters of *Fusarium* species in sections *Liseola* and *Elegans* and similar species. Mycologia 82:99-106.

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.

Olsson, K. 1989. Relationship between pectolytic enzyme activity and rot development in potato tubers inoculated with *Fusarium solani* f.sp. *coeruleum.* Journal of Phytopathology 124:225-235.

Samac, D.A. and S.A. Leong. 1989. Disease development in *Cucurbita maxima* (squash) infected with *Fusarium solani* f.sp. *cucurbitae.* Canadian Journal of Botany 67:3486-3489.

Schippers, B. and K.M. Old. 1974. Factors affecting chlamydospore formation by *Fusarium solani* f.sp. *cucurbitae* in pure culture. Soil Biology and Biochemistry 6:153-160.

Schroth, M.N. and W.C. Snyder. 1961. Effect of host exudates on chlamydospore germination of the bean root rot fungus, *Fusarium solani* f.sp. *phaseoli.* Phytopathology 51:390-393.

Schuerger, A.C. and D.J. Mitchell. 1992. Effects of temperature and hydrogen ion concentration on attachment of macroconidia of *Fusarium solani* f.sp. *phaseoli* to mung bean roots in hydroponic nutrient solutions. Phytopathology 82:1311-1319.

Schuerger, A.C. and D.J. Mitchell. 1993. Influence of mucilage secreted by macroconidia of *Fusarium solani* f.sp. *phaseoli* on spore attachment to roots of *Vigna radiata* in hydroponic nutrient solution. Phytopathology 83:1162-1170.

Shrimpton, G. and F. Williams. 1989. Benomyl drenches: no control for *Fusarium* or *Cylindrocarpon* caused root rot of container-grown conifer seedlings. Canada-British Columbia Economic & Regional Development Agreement. Memo No. 128. 1p.

Snyder, W.C. 1981. Introduction. In:. Nelson, P.E., T.A. Toussoun and R.J. Cook (eds.). *Fusarium*: Diseases, Biology, and Taxonomy. The Pennsylvania State University Press, University Park. pp. 3-8.

Sturz, A.V. and C.C. Bernier. 1991. Fungal communities in winter wheat roots following crop rotations suppressive and nonsuppressive to take-all. Canadian Journal of Botany 69:39-43.

Summerell, B.A., C.A. Rugg and L.W. Burgess. 1993. Mycogeography of *Fusarium*: survey of *Fusarium* species associated with forest and woodland communities in north Queensland, Australia. Mycological Research 97:1015-1019.

Sutherland, J.R. and E. van Eerden. 1980. Diseases and insect pests in British Columbia forest nurseries. British Columbia Ministry of Forests/Canadian Forestry Service, Joint Report No. 12. 55p.

Vaartaja, O. 1967. Damping-off pathogens in South Australian nurseries. Phytopathology 57:765-768.

Vaartaja, O. and M. Bumbieris. 1967. Organisms associated with root rots of conifers in South Australian nurseries. Plant Disease Reporter 51:473-476.

Vaartaja, O. and W.H. Cram. 1956. Damping-off pathogens of conifers and of *Caragana* in Saskatchewan. Phytopathology 46:391-397.

Viljoen, A., M.J. Wingfield, W.F.O. Marasas and T.A. Coutinho. 1995. Characteristics of *Fusarium* isolates from gladiolus corms pathogenic to pines. Plant Disease 79:1240-1244.

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