NURSERY DISEASE NOTES USDA FOREST SERVICE

NORTHERN REGION
FOREST HEALTH PROTECTION

No. 148 February 2003

ACCUMULATION OF ROOT PATHOGENS ON BARE ROOT DOUGLAS-FIR AND ENGLEMANN SPRUCE SEEDLINGS INDUCES FOLIAR CHLOROSIS AFTER THREE GROWING SEASONS AT THE USDA FOREST SERVICE NURSERY, COEUR D'ALENE, IDAHO

R.L. James
Plant Pathologist

ABSTRACT

Scattered bare root Douglas-fir and Engelmann spruce seedlings grown for three years at the USDA Forest Service Nursery, Coeur d'Alene, Idaho were chlorotic and appeared diseased. Douglas-fir seedlings had moderate levels of root colonization by *Fusarium* spp. (primarily *F. oxysporum*) but extensive root colonization by *Cylindrocarpon destructans*. Engelmann spruce seedlings were extensively colonized by *F. oxysporum*, but had only low levels of colonization by *C. destructans*. Several other species of *Fusarium* were isolated at low frequencies from both conifer species. Four different morphotypes of *F. oxysporum* were isolated from seedlings; pathogenicity and/or virulence of different isolates may not necessarily be related to morphotype. Two *Pythium* spp. (*P. irregulare* and *P. ultimum*) were commonly isolated from roots of bare root Douglas-fir seedlings; much lower levels of these fungi were isolated from Englemann spruce seedlings. *Phytophthora* spp. were isolated at very low levels from both species. Results indicated that *F. oxysporum*, *C. destructans* and *P. irregulare* were the most important potentially-pathogenic fungi contributing to production of chlorotic foliage on sampled seedlings.

INTRODUCTION

Bare root conifer seedlings are routinely grown for two years at the USDA Forest Service Nursery in Coeur d'Alene, Idaho. At the end of the second growing season, seedlings are usually lifted and placed in cold storage prior to shipment to the field. On rare occasions seedlings have to be held in production fields for an additional year due to unanticipated problems, such as inability to outplant seedlings as planned. To grow seedlings another year, they must often be periodically root pruned and sometimes top pruned as well to ensure that they do not become too large for easy extraction from the nursery or outplanting. Pruning often results in extensive wounding that may enhance disease problems.

Portions of recent crops of bareroot Douglas-fir (*Pseudotsuga menziesii* Franco var. *glauca* [Mayr] Sudw.) and Englemann spruce (*Picea engelmanni* Parry) seedlings at the Coeur d'Alene Nursery were held for a third growing season because of changes in outplanting schedules. By the end to the third growing season, a significant proportion of these seedlings displayed foliar chlorosis (figures 1 and 2); some scattered necrosis was also evident. These seedlings had undergone several root prunings to limit growth of root systems. Since root-pathogenic fungi often elicit chlorotic foliage on infected bare root conifer seedlings (Bloomberg 1971; James et al. 1991), an evaluation was conducted to quantify level of these fungi on roots.

MATERIALS AND METHODS

In the fall at the end of the third growing season, 17 chlorotic Douglas-fir and 25 chlorotic Engelmann spruce seedlings were randomly selected for sampling. Seedlings were carefully extracted from bare root beds to ensure that most of their roots systems were collected. Seedling roots were washed thoroughly to remove adhering soil particles. Selected lateral roots were detached from root systems by cutting with a sterile knife. Detached roots were placed in sterile water for several minutes, and cut into pieces approximately 5 mm in length. Root pieces were surface sterilized in a 10% bleach solution (0.525% aqueous sodium hypochlorite), rinsed in sterile water, and placed on each of two selective agar media. One medium was selective for *Fusarium* and closely-related species (Komada 1975); the other medium was selective for water mold (Oomycete) organisms such as *Pythium* and *Phytophthora* and consisted of V-8 juice agar amended with the antibiotics pimaricin, rifamycin, ampicillin, and the fungicide pentachloronitrobenzene (James et al. 1990, 1996; Stone et al. 1995). Plates of Komada's medium were incubated under diurnal cycles of cool, fluorescent light at about 24°C for 7-10 days. Selected isolates of emerging fungi were transferred to potato dextrose agar (PDA) and carnation leaf agar (Fisher et al. 1982) to facilitate identification. *Fusarium* and *Cylindrocarpon* spp. were identified using the taxonomy of Nelson et al. (1983) and Booth (1966), respectively. *Pythium* spp. were identified using the taxonomy of Waterhouse (1968). Percentage of root

pieces colonized by *Fusarium*, *Cylindrocarpon*, and *Pythium* species were calculated. In addition, isolates of *Fusarium oxysporum* were categorized on the basis of their different morphology on PDA (morphotypes) in order to facilitate characterization of this diverse taxon (Gordon and Martyn 1997)

Figure 1. Chlorotic bare root 3-0 Douglas-fir seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho. Necrotic seedlings are indicated by an arrow.

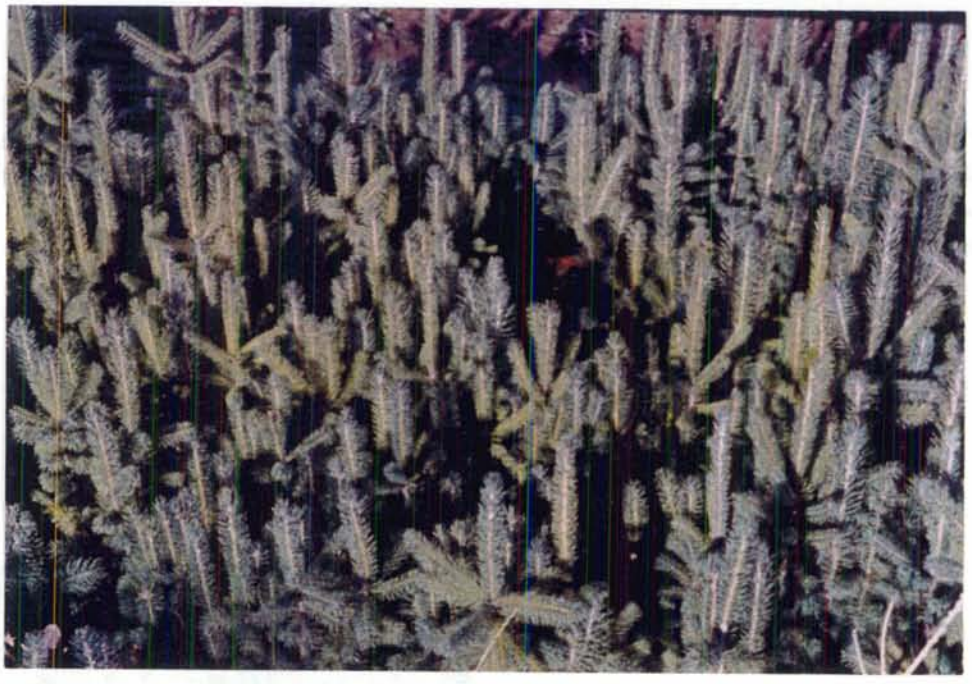

Figure 2. Chlorotic bare root 3-0 Engelmann spruce seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

RESULTS

Fusarium spp. were isolated from about 56% and 95% of the sampled root pieces of Douglas-fir and Engelmann spruce, respectively (tables 1 and 2). Levels of *Fusarium* colonization varied much more on Douglas-fir than on Engelmann spruce seedling roots, although all sampled species of both species were infected. By far the most common species isolated was *F. oxysporum* Schlecht. This species comprised four distinctmorphotypes (table 3) which differed on the basis of their colony morphology on PDA. All morphotypes had cultural characteristics descriptive of *F. oxysporum* including production of globose chlamydospores (either singly, in pairs, or small groups), microconidia borne on short, unbranched monophialides, and characteristic macroconidia. Morphotypes were mostly delimited on the basis of extent and color of aerial mycelium, pigmentation, and presence or absence of pionnotal sporodochia. For example, morphotype 1, which was the most commonly-isolated type of *F. oxysporum*, produced abundant white aerial mycelium and limited violet pigmentation within the agar, without pionnotal sporodochia. Morphotype 2 was fairly similar but with slightly violet-pink aerial mycelium and more intense violet pigmentation. Morphotypes 3 and 4 had little or no aerial mycelium with presence (morphotype 3) and absence (morphotype 4) of pionnotal sporodochia.

Four other *Fusarium* species were isolated at much lower levels: *F. avenaceum* (Fr.) Sacc., *F. equiseti* (Corda) Sacc., *F. solani* (Mart.) Appel & Wollenw., and *F. sporotrichioides* Sherb. *Cylindrocarpon* spp. (primarily *C. destructans* (Zins.) Scholten and

much lower levels of *C. tenue* Bugn.) were isolated at high levels from roots of Douglas-fir seedlings (table 1), but at much lower levels from Engelmann spruce seedlings (table 2).

Oomycetes were also isolated frequently from Douglas-fir (table 4) and at lower levels from Engelmann spruce (table 5). Two species of *Pythium* (*P. irregulare* Buisman and *P. ultimum* Trow.) were present on both Douglas-fir and Engelmann spruce seedlings. *Pythium irregulare* was isolated at much higher levels than *P. ultimum* on both conifer species. Unidentified *Phytophthora* spp. were isolated at very low levels from both conifer species (tables 4 and 5).

DISCUSSION

Relatively high levels of root colonization by *Fusarium* and *Cylindrocarpon* spp. were detected on the roots of chlorotic 3-0 bare root Douglas-fir and Engelmann spruce seedlings at the Coeur d'Alene Nursery. In addition, Oomycete pathogens in the genus *Pythium* were isolated at fairly high levels from nearly all Douglas-fir and about half of the sampled Engelmann spruce seedlings. Therefore, it appears that all three groups of fungi significantly contributed to development of the chlorotic foliage disease symptoms.

Table 1. Occurrence of *Fusarium* and *Cylindrocarpon* spp. on roots of chlorotic 3-0 bare root Douglas-fir seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho[1].

Seedling Number	*Fusarium*[2]					*Cylindrocarpon*[3]	
	FOXY	FAVE	FEQU	FSPO	ALL	CYDE	CYTE
1	33.3	6.7	0	0	40.0	80.0	0
2	66.7	26.7	0	0	93.3	33.3	0
3	26.7	13.3	0	0	40.0	86.7	6.7
4	13.3	20.0	0	0	33.3	86.7	0
5	53.3	0	0	0	53.3	66.7	0
6	40.0	6.7	0	0	46.7	66.7	0
7	46.7	26.7	13.3	0	86.7	53.3	0
8	46.7	0	6.7	0	53.3	73.3	0
9	33.3	6.7	0	0	40.0	80.0	0
10	26.7	0	0	0	26.7	93.3	0
11	40.0	20.0			60.0	86.7	0
12	40.0	0	0	0	40.0	80.0	0
13	100.0	20.0	0	0	100.0	66.7	0
14	53.3	6.7	0	6.7	66.7	93.3	0
15	60.0	6.7	0	0	66.7	73.3	0
16	66.7	0	0	0	66.7	60.0	0
17	26.7	20.0	0	0	46.7	80.0	0
Average	45.5	10.6	1.2	0.4	56.5	74.1	0.4

[1] Values in table are percent of root pieces (15 sampled per seedling) colonized by the appropriate fungus.
[2] *Fusarium* spp.: FOXY = *F. oxysporum;* FAVE = *F. avenaceum*; FEQU = *F. equiseti*; FSPO = *F. sporotrichioides.*
[3] *Cylindrocarpon* spp.: CYDE = *C. destructans*; CYTE = *C. tenue.*

Table 2. Occurrence of *Fusarium* and *Cylindrocarpon* spp. on roots of chlorotic 3-0 bare root Englemann spruce seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho[1].

Seedling Number	*Fusarium*[2]						*Cylindrocarpon*[3]	
	FOXY	FAVE	FEQU	FSOL	FSPO	ALL	CYDE	CYTE
1	66.7	6.7	6.7	0	0	80.0	20.0	6.7
2	86.7	6.7	0	0	0	93.3	13.3	0
3	100.0	13.3	0	0	0	100.0	0	0
4	86.7	6.7	0	0	0	93.3	13.3	0
5	100.0	0	0	0	0	100.0	13.3	0
6	100.0	0	0	0	0	100.0	6.7	0
7	73.3	0	0	0	0	73.3	33.3	0
8	100.0	0	6.7	0	0	100.0	13.3	0
9	100.0	0	6.7	0	0	100.0	20.0	0
10	100.0	0	0	0	0	100.0	6.7	0
11	93.3	0	13.3	0	0	100.0	6.7	0
12	100.0	6.7	0	6.7	0	100.0	13.3	0
13	100.0	0	6.7	0	0	100.0	6.7	0
14	93.3	0	0	0	0	93.3	13.3	0
15	93.3	6.7	0	6.7	0	100.0	6.7	6.7
16	100.0	6.7	0	0	0	100.0	0	0
17	100.0	6.7	6.7	0	0	100.0	6.7	0
18	80.0	6.7	0	0	6.7	93.3	20.0	0
19	100.0	0	0	0	0	100.0	13.3	0
20	93.3	0	0	0	6.7	100.0	20.0	0
21	73.3	0	0	0	0	73.3	26.7	0
22	100.0	0	0	0	0	100.0	13.3	0
23	73.3	0	0	0	0	73.3	33.3	0
24	80.0	6.7	6.7	0	0	93.3	13.3	0
25	100.0	6.7	0	0	0	100.0	20.0	0
Average	91.7	3.2	2.1	0.5	0.5	94.7	14.1	0.5

[1] Numbers in table are percent of root pieces (15 sampled per seedling) colonized with appropriate fungus.
[2] *Fusarium* spp.: FOXY = *F. oxysporum*; FAVE = *F. avenaceum*; FEQU = *F. equiseti*; FSOL = *F. solani*; FSPO = *F. sporotrichioides*.
[3] *Cylindrocarpon* spp.: CYDE = *C. destructans*; CYTE = *C. tenue*.

Table 3. Distribution of *Fusarium oxysporum* morphotypes on roots of chlorotic 3-0 bare root Douglas-fir and Engelmann spruce seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Species	Isolates Sampled	Morphotype 1 (%)	Morphotype 2 (%)	Morphotype 3 (%)	Morphotype 4 (%)
Douglas-fir	115	61.7	34.9	1.7	1.7
Engelmann Spruce	363	66.4	33.6	0	0
Both Species	478	65.3	33.9	0.4	0.4

Table 4. Occurrence of *Pythium* and *Phytophthora* spp. on roots of chlorotic 3-0 bare root Douglas-fir seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Seedling Number	*Pythium*			*Phytophthora* spp.
	P. irregulare	*P. ultimum*	All *Pythium*	
1	20	20	40	0
2	20	40	60	0
3	40	0	40	0
4	20	0	20	0
5	60	0	60	0
6	40	20	60	0
7	60	0	60	0
8	40	20	60	20
9	40	0	40	0
10	20	0	20	0
11	0	0	0	0
12	40	0	40	0
13	40	0	40	0
14	20	0	20	0
15	20	20	40	0
16	60	0	60	0
17	60	0	60	0
Average	35.3	7.0	42.3	1.2

Fusarium and *Cylindrocarpon* spp. are two groups of fungi that commonly colonize roots of conifer seedlings grown in bare root forest nurseries (Bloomberg 1971, 1973; Dahm and Strzelczyk 1967; Edmonds and Heather 1973; James et al. 1991). These fungi can be detected at relatively high levels on roots of diseased seedlings periodically throughout typical growing cycles (Bloomberg 1971; James et al. 1991) and healthy-appearing seedlings at the time of lifting (Bloomberg 1966; James and Gilligan 1988). Isolates of both groups of these fungi are capable of either eliciting disease symptoms on infected seedlings (pathogenic) or acting as saprophytes or endophytes within infected seedlings (Bloomberg 1966; Booth 1966; James et al. 1991). *Fusarium* and *Cylindrocarpon* can respond to changes in host stresses and become pathogenic when and initiate disease symptoms when stresses reach critical levels (Elad and Baker 1985; Harling et al. 1988; James 1994). Factors contributing to seedling stress may include moisture excesses and deficiencies (Brownell and Schneider 1985; Clayton 1923a; James et al. 1994), nutrient imbalances (Bloom and Walker 1955; Duffy and Defago 1999; James 1997), temperature extremes (Bloomberg 1973, 1979; Fisher and Toussoun 1983) and enhanced competition for growing space (and associated competition for moisture and nutrients) resulting from high seedling densities.

Table 5. Occurrence of *Pythium* and *Phytophthora* spp. on roots of chlorotic 3-0 bare root Engelmann spruce seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Seedling Number	*Pythium*			*Phytophthora* spp.
	P. irregulare	*P. ultimum*	All *Pythium*	
1	20	0	20	0
2	0	0	0	20
3	0	0	0	0
4	20	0	20	0
5	0	20	20	0
6	0	0	0	0
7	0	0	0	0
8	40	0	40	0
9	40	0	40	0
10	0	0	0	40
11	20	0	20	0
12	20	0	20	0
13	0	0	0	0
14	40	0	40	0
15	20	0	20	0
16	40	0	40	0
17	0	0	0	0
18	0	0	0	0
19	20	0	20	0
20	0	0	0	0
21	20	20	40	0
22	20	0	20	0
23	0	0	0	0
24	0	0	0	0
25	0	0	0	0
Average	12.8	1.6	14.4	2.4

Seedling roots are infected with both *Fusarium* and *Cylindrocarpon* spp. throughout their growing cycle in bare root beds, although most infection occurs during the first growing season (Bloomberg 1981; James 1996). Seedlings can become initially infected shortly after seed germination (Bloomberg 1971; James et al. 1991) from inoculum residing either on seed or within the soil. Level of root infection is related to inoculum levels and aggressiveness of fungal populations. When disease occurs, most of it develops during the first growing season either shortly after germination (damping-off) or periodically throughout the first year, especially when ambient temperatures become high during the summer (Bloomberg 1973, 1979; Buxton et al. 1962; Clayton 1923b). By the end of the first growing season, most root-associated disease that is going to affect the crop has occurred (Bloomberg 1981; Enebak et al. 1990). During the

second and subsequent growing seasons, disease is less common, although root infection by potentially-pathogenic isolates of either *Fusarium* or *Cylindrocarpon* can continue to occur (Enebak et al. 1990; James et al. 1991).

Although relatively large portions of seedling root systems may be colonized by *Fusarium* and *Cylindrocarpon* when seedlings are lifted for outplanting, these fungi usually do not adversely affect outplanting performance (Dumroese et al. 1993, 2000). This is primarily because these nursery-colonizing organisms are replaced by other mycoflora once seedlings are outplanted on forest sites (Dumroese et al. 1993, 2000). Resident populations of *Fusarium* and *Cylindrocarpon* are detected at increasingly lower levels during the months following ouplanting. Important replacement fungi include ecto-mycorrhizal symbionts and common saprophytes that reside in forest soil (Dumroese et al. 1993, 2000), some of which may actually be antagonistic toward pathogens (Chakravarty et al. 1990, 1991; Duchesne et al. 1989).

Fusarium oxysporum was isolated more frequently from the roots of both Douglas-fir and Engelmann spruce seedlings than any of the other potentially-pathogenic fungi. This fungus taxon probably comprises several closely-related species that produce similar morphological characteristics (Gordon and Martyn 1997; Gordon and Okamoto 1992). Genetic diversity with the *F. oxysporum* complex is extreme (Gordon and Martyn 1997; Kistler 1997) and several molecular markers have been identified that can be used to separate genetically different isolates (Edel et al. 1995; Gordon and Okamoto 1992; Kim et al. 1992; Kistler et al. 1987). Some of these markers have successfully delineated pathogenic from saprophytic isolates that may occur on several different host species (Gordon and Martyn 1997; Gordon and Okamoto 1992).

Isolates of *F. oxysporum* obtained from roots of conifer seedlings or nursery soil can often be separated on the basis of the different morphologies (morphotypes) on standard laboratory growth media (Bloomberg 1976; Hartley and Merrill 1918; James et al. 1989). Since *F. oxysporum* populations are usually represented by several different clones (the species does not undergo sexual recombination but rather only reproduces asexually), these different morphotypes may represent individual clones. Unfortunately, different morphotypes are not often correlated with genetic differences that influence pathogenicity or virulence (Gordon and Martyn 1997). Therefore, the only way to differentiate isolates on the basis of their ability to incite disease is to either subject them to genetic analysis which requires sophisticated and costly equipment or evaluate their ability to elicit disease in carefully-controlled pathogenicity tests. Such tests are expensive and very time consuming.

Pythium spp. are quite common at the Coeur d'Alene Nursery and sometimes elicit serious diseases of bare root seedlings, particularly under conditions of prolonged high soil moisture (James 1982; James et al. 1991). Both species isolated from seedling roots (*P. irregulare* and *P. ultimum*) are common soil inhabitants and often associated with the roots of chlorotic seedlings (James 2000).

It is suspected that disease levels would have been much less if these Douglas-fir and Engelmann spruce seedlings had been lifted and planted as initially scheduled. However, keeping them in dense seedbeds for an additional year coupled with several root pruning operations provided ideal conditions for isolates of *Fusarium*, *Cylindrocarpon*, and *Pythium* colonizing root systems to become pathogenic and initiate root deterioration, resulting in above-ground disease symptoms. Previous work (James and Perez 1999) has shown that all of these fungi readily colonize residual roots in soil left either after lifting the seedling crop or following root pruning. These important sources of organic matter in the soil tends to stimulate population explosions of these potential pathogens.

Because the field where disease occurred had been fumigated prior to sowing with dazomet, it is suspected that populations of *Fusarium*, *Cylindrocarpon*, and *Pythium* were initially quite low. However, over time these populations likely increased, probably primarily from contamination from surrounding non-fumigated fields or on infected seed. Populations were sufficient to initiate extensive root infection after two growing seasons. Therefore, it appears that the longer bareroot seedlings are kept growing in nursery fields, the greater the likelihood of impact by important root pathogens, even in fumigated ground. As a result, it may be preferable to transplant 2-0 seedlings in other portions of the nursery to reduce seedling density and perhaps limit stress than seedlings undergo during their third growing season.

LITERATURE CITED

Bloom, J.R. and J.C. Walker. 1955. Effect of nutrient sprays on Fusarium wilt of tomato. Phytopathology 45:443-444.

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

Bloomberg, W.J. 1971. Diseases of Douglas-fir seedlings caused by *Fusarium oxysporum*. Phytopathology 61:467-470.

Bloomberg, W.J. 1973. Fusarium root rot of Douglas-fir seedlings. Phytopathology 63:337-341.

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

Bloomberg, W.J. 1979. Model simulation of infection of Douglas-fir seedling by *Fusarium oxysporum*. Phytopathology 69:1072-1077.

Bloomberg, W.J. 1981. Diseases caused by *Fusarium* in forest nurseries. *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. 178-187.

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

Brownell, K.H. and R.W. Schneider. 1985. Roles of matric and osmotic components of water potential and their interaction with temperature in the growth of *Fusarium oxysporum* in synthetic media and soil. Phytopathology 75:53-57.

Buxton, E.W., I. Sinha and V. Ward. 1962. Soil-borne diseases of Sitka spruce seedlings in a forest nursery. Transactions of the British Mycological Society 45:433-488.

Charkravarty, P., R.L. Peterson and B.E. Ellis. 1990. Integrated control of *Fusarium* damping-off in red pine seedlings with the ectomycorrhizal fungus Paxillus involutus and fungicides. Canadian Journal of Forest Research 20:1283-1285.

Chakravarty, P., R.L. Peterson and B.E. Ellis. 1991. Interaction between the ectomycorrhizal fungus *Paxillus involutus*, damping-off fungi and *Pinus resinosa* seedlings. Journal of Phytopathology 132:207-218.

Clayton, E.E. 1923a. The relation of soil moisture to the *Fusarium* wilt of the tomato. American Journal of Botany 10:133-147.

Clayton, E.E. 1923b. The relation of temperature to the *Fusarium* wilt of the tomato. American Journal of Botany 10:71-88.

Dahm, H. and E. Strzelczyk. 1987. Effect of pH, temperature and light on the pathogenicity of *Cylindrocarpon destructans* to pine seedlings in associative cultures with bacteria and actinomycetes. European Journal of Forest Pathology 17:141-148.

Duchesne, L.C., S.E. Campbell, H. Koehler and R.L. Peterson. 1989. Pine species influence suppression of Fusarium root rot by the ectomycorrhizal fungus *Paxillus involutus*. Symbiosis 7:139-148.

Duffy, B.K. and G. Defago. 1999. Macro- and microelement fertilizers influence the severity of Fusarium crown and root rot of tomato in a soilless production system. HortScience 34:287-291.

Dumroese, R.K., R.L. James and D.L. Wenny. 1993. *Fusarium* root infection of container-grown Douglas-fir: effects 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.

Edel, V., C. Steinberg, I. Avelange, G. Laguerre and C. Alabouvette. 1995. Comparison of three molecular methods for the characterization of *Fusarium oxysporum* strains. Phytopathology 85:579-585.

Edmonds, R.L. and W.A. Heather. 1973. Root diseases in pine nurseries in the Australian Capital Territory. Plant Disease Reporter 57:1058=1062.

Elad, Y. and R. Baker. 1985. The role of competition for iron and carbon in suppression of chlamydospore

germination by *Fusarium* spp. by *Pseudomonas* spp. Phytopathology 75:1053-1059.

Enebak, S.A., M.A. Palmer and R.A. Blanchette. 1990. Managing soilborne pathogens of white pine in a forest nursery. Plant Disease 74:194-198.

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.

Fisher, N.L. and T.A. Toussoun. 1983. Symptom response and colonization as measures of resistance in chrysanthemum cultivars inoculated with *Fusarium oxysporum* f.sp. *chrysanthemi*. Plant Disease 67:376-378.

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

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

Harling, R., G.S. Taylor, P. Matthews and A.E. Arthur. 1988. The effect of temperature on symptom expression and colonization in resistant and susceptible carnation cultivars infected with *Fusarium oxysporum* f.sp. *dianthi*. Journal of Phytopathology 121:103-117.

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

James, R.L. 1982. Pythium root disease of Douglas-fir and grand fir seedlings at the Coeur d'Alene Nursery, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Report 82-10. 10p.

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 Forest and Range Experiment Station. General Technical Report GTR-184. pp. 29-34.

James, R.L. 1996. Root disease of 1-0 bareroot seedlings - USDA Forest Service Lucky Peak Nursery, Boise, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Report 96-4. 10p.

James, R.L. 1997. Effects of fertilizer on selected potential plant pathogens in bareroot forest nurseries. *In*: Haase, D.L. and R. Rose (eds.). Symposium Proceedings: Forest Seedling Nutrition from the Nursery to the Field. Nursery Technology Cooperative. Oregon State University, Corvallis. pp. 27-39.

James, R.L. 2000. Effects of a 2-year fallow period on soil populations of *Fusarium, Trichoderma* and *Pythium* species after incorporating corn plant residues - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Report 0-17. 11p.

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., R.K. Dumroese and D.L. Wenny. 1989. Occurrence, characteristics, and descriptions of *Fusarium* 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. 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 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. and C.J. Gilligan. 1986. Root diseases of western white pine transplants at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Report 86-11. 8p.

James, R.L. and C.J. Gilligan. 1988. 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., 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., 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. and R. Perez. 1999. 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.

Kim, D.H., R.D. Martyn and C.W. Magill. 1992. Restriction fragment length polymorphism groups and the physical map of mitochondrial DNA from *Fusarium oxysporum* f.sp. *niveum*. Phytopathology 82:348-353.

Kistler, H.C. 1997. Genetic diversity in the plant-pathogenic fungus *Fusarium*

oxysporum. Phytopathology 87:474-479.

Kistler, H.C., P.W. Bosland, U. Benny, S. Leong and P.H. Williams. 1987. Relatedness of strains of *Fusarium oxysporum* from crucifers measured by examination of mitochondrial and ribosomal DNA. Phytopathology 77:1289-1293.

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.

Stone, J.K., D.M. Hildebrand, R.L. James, S.M. Frankel and D.S. Gemandt. 1995. Alternatives to methyl bromide for control of soil-borne diseases in bare root forest nurseries. In: Proceedings: Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions. November 6-8, 1995, San Diego, CA. Methyl Bromide Alternatives Outreach, U.S. Environmental Protection Agency and U.S. Department of Agriculture. pp. 77-1 – 77.4.

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.