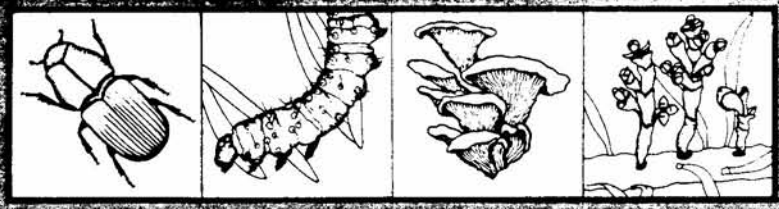
Forest Health Protection



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WESTERN WHITE PINE SEEDLING MORTALITY USDA FOREST SERVICE NURSERY, COEUR D'ALENE, IDAHO

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ABSTRACT

Western white pine seedlot 7173 was harvested from the Coeur d'Alene Nursery seed orchard in 1993 and processed at the nursery. Subsequent bareroot and container seedling crops using this seedlot were marked by extremely poor germination. Potentially pathogenic fungi did not extensively colonize seed. However, diseased seedlings were always colonized with *Fusarium* spp., particularly *F. oxysporum* in bareroot beds and *F. culmorum* and *F. avenaceum* in containers. Levels of potentially pathogenic *Fusarium* and *Pythium* in soil were not high where bareroot seedling production was poor. Populations of these fungi in portions of the field which had an incorporated corn cover crop were higher than portions without a cover crop. Ratios of *Trichoderma* (potentially antagonistic) to *Fusarium* (potentially pathogenic) were low throughout the field. Although *Fusarium* species may be important in limiting seedling production, they were not the major cause of poor seed germination and reduced seedling establishment. Other unidentified problems, perhaps related to seed dormancy, may have been more important.

INTRODUCTION

Western white pine (*Pinus monticola* Dougl.) is a very important component of forest ecosystems in the inland Pacific Northwest.

This species once comprised a much larger proportion of forest stands than currently, but was devastated by the introduced white pine blister rust (*Cronartium ribicola*), bark beetle attacks, and extensive logging (Hagle and others 1989). Regenerating forest sites that once supported higher levels of white pine has become a high priority for forest managers. Large numbers of blister rust-resistant seedling stock are produced at several nurseries, including the USDA Forest Service Nursery in Coeur d'Alene, Idaho. Both bareroot and container white pine stock are grown.

An operational seed orchard producing blister-rust resistant seed is located on site. Cones are collected annually and processed at the nursery. Seed from lot 7173, harvested from the orchard in 1993, resulted in bareroot seedling stands that were very sparse with extensive mortality of emerged seedlings by the end of the first growing season (figure

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1). Seedling production from the field was much less than growers anticipated. Similar results occurred with the same seedlot grown as a container crop (figure 2). Many empty cavities were evident even though four seeds were sown per cavity.

An evaluation was conducted to determine possible associations between potentially pathogenic fungi and occurrence of seedling mortality in both bareroot beds and within containers.

MATERIALS AND METHODS

The southern part of field 9 at the nursery was used to grow a western larch early selection trial which was terminated in fall of 1992. This field was fallowed during winter of 1992-1993. During the 1993 growing season, a cover/green manure crop of sweet and field corn was grown to improve tilth and increase organic matter. The crop was chopped and incorporated into soil in mid-summer. In late August, the field was fumigated with dazomet (350 lbs/A; 392.3 kg/ha) and fallowed over winter. Western white pine seeds from seedlot 7173, collected from the Coeur d'Alene Nursery seed orchard and processed on site, were rinsed 48 hours in running water before placement in cold-moist stratification for 120 days. During stratification, seeds were rinsed each week in running water (usually for a couple of hours). In April 1994, part of the seedlot was planted in soil with the incorporated corn crop and part in soil fallowed during 1993.

In October, 1994, 100 soil samples were systematically collected throughout field 9. Sixty samples were from portions of the field with the incorporated corn crop and 40 from the fallowed portion. Samples were collected along east-west transects at 20-feet (6.1-m) intervals (corresponding to irrigation riser locations). Ten soil core samples were collected per transect at a depth of about 15 cm. Standard soil dilution techniques were used to determine populations of two groups of potential plant pathogenic fungi: *Fusarium* and *Pythium* spp. (James and others 1990). In addition, populations of *Trichoderma* were estimated when *Fusarium* populations were determined. Soil was initially sieved (2.4 mm sieve) to remove rocks, pieces of undecomposed organic matter, and soil aggregations. From each sample, a 5 g subsample was dried at about 100°C for at least 24 hours or until sample weight had stabilized (all excess moisture removed). Oven-dry weight was then calculated to provide a standard for comparison. For assay of *Fusarium* and *Trichoderma* populations, 0.5 g of field-moist soil was combined with 100 ml of 0.3 percent water agar and thoroughly mixed. One ml of solution was placed on each of three plates of selective agar medium (Komada 1975) and spread uniformly. Plates were incubated 5 days at about 24°C under diurnal cycles of cool, fluorescent light. *Fusarium* and *Trichoderma* colonies were identified by their morphology on the selective medium and populations determined. Ratios of *Trichoderma* to *Fusarium* were calculated for a rough estimate of potential soil suppressiveness to root pathogens. For assay of *Pythium* populations, 5.0 g of soil was combined with 100 ml of 0.3 percent water agar. One ml of solution was placed on each of three plates of another selective medium consisting of V-8 juice agar amended with rose bengal, pimaricin, rifamycin, ampicillin, and pentachloronitrobenzene (James and others 1990). Plates were incubated 3 days in the dark at about 24°C. *Pythium* colonies were identified based on their diameter after 3 days (15-20 mm), their feathery margin, and whether they grew within, rather than superficially on, the agar surface. Populations of *Fusarium*, *Trichoderma*, and *Pythium* were expressed as colony-forming units per g of soil.

When soil was collected, seedlings were randomly selected from throughout affected seedbeds for analysis of potentially pathogenic fungi colonizing roots. A total of 76 seedlings displaying typical root disease symptoms (foliar chlorosis and necrosis and stunting) were sampled. Seedling roots were washed to remove adhering soil, cut into segments 10 mm long, surface sterilized in a 10 percent bleach solution (0.525 percent aqueous sodium hypochlorite), rinsed in sterile water, and placed on selective agar medium (Komada 1975). Plates were incubated as described above for *Fusarium* spp. Selected isolates were transferred to potato dextrose agar and carnation leaf agar (Fisher and others 1983) for identification using the taxonomic methods of Nelson and others (1983).

The 1995 container crop of white pine was sown with the same seedlot (7173) as the bareroot crop. Seeds were sown in January and by mid-February seedling establishment and filled cells were much below normal (figure 2). Forty seedlings with typical post-emergence damping-off symptoms were collected. Concurrently, 59 ungerminated seed and/or discarded seedcoats with distinctive fungal sporodochia (figure 3) were collected from tops of cavi-

ties. Seedling roots and seed/seedcoats were placed on a selective medium (Komada 1975) and incubated as described above. Selected *Fusarium* isolates were identified.

Seeds from lot 7173 were sampled for contamination with *Fusarium* spp. which might be involved in reducing germination and/or inciting pre- and post-emergence damping-off. Seeds were sampled at three times during the processing/sowing sequence: (1) immediately after extraction from cones, (2) during cold temperature storage (prior to stratification), and (3) at sowing. Four hundred seeds were randomly selected for the first two samples and 800 for the third sample. Seeds were placed directly on a selective agar medium (Komada 1975) (20 seeds per plate) and incubated as described above. Selected *Fusarium* isolates were identified.

Figure 1. Bareroot 1-0 western white pine seedlings within field 9, USDA Forest Service Nursery, Coeur d'Alene, Idaho. Seedling stands were sparse with high mortality of established seedlings.

Figure 2. Container-grown western white pine seedlings, USDA Forest Service Nursery, Coeur d'Alene, Idaho. Many empty cells were evident despite sowing four seeds per cell.

Figure 3. Orange *Fusarium* sporodochia on seedcoat of western white pine, USDA Forest Service Nursery, Coeur d'Alene, Idaho. *Fusarium* contaminating the seedcoat attacked the primary root shortly after emergence.

RESULTS AND DISCUSSION

Populations of *Fusarium*, *Trichoderma* and *Pythium* spp. all increased dramatically following incorporation of the corn cover crop into field 9 (table 1). Soil microorganism populations tend to increase following organic matter addition, such as incorporating a cover crop (Hamm and Hansen 1990; James and others 1996). This population buildup may be fairly non-selective. Average soil populations of *Fusarium* were variable, but usually not very high. Portions of the field with incorporated corn cover crop generally had higher populations than the fallowed portion. *Trichoderma* spp., which may be antagonistic toward soil-borne pathogens including *Fusarium* spp. (Papavizas 1985), were similarly affected by presence of the incorporated corn crop. *Pythium* populations were also higher in portions of the field with the incorporated cover crop (table 1). Although the entire field was fumigated with dazomet before sowing, soil populations of the three groups of fungi assayed were not entirely eliminated by fumigation. Dazomet fumigation does not always penetrate deeply into soil and some fungi may escape (Hoffman and Williams 1988; James and others 1990). Residual fungal propagules after fumigation were sufficient to elicit disease following sowing of white pine seed.

All diseased bareroot seedlings sampled were extensively colonized with *Fusarium*, primarily *F. oxysporum* Schlecht. (table 2). This species was also most often isolated from soil. *Fusarium oxysporum* has frequently been isolated from soil (James and others 1990, 1991) and both diseased (James 1983, 1987a) and non-diseased (James and Gilligan 1988) seedlings at the nursery. Although three other *Fusarium* spp. [*F. acuminatum* Ell. & Ev., *F. solani* (Mart.) Appel & Wollenw., and *F. equiseti* (Corda) Sacc.] were isolated at low levels from soil, none of these fungi were detected on roots of diseased seedlings. Therefore, the major pathogen associated with seedling mortality was *F. oxysporum*.

Seven different *Fusarium* spp. were isolated from white pine seed (table 3). However, even the most common (*F. culmorum* (W. G. Smith) Sacc.) occurred at fairly low levels. Overall seed infection with *Fusarium* spp. was low, with the greatest amounts detected at sowing. *Fusarium* levels on seed taken directly from cones was very low (2.5 percent); low levels were also detected on cold stored seeds. Apparently, *Fusarium* slightly increased during stratification and pre-sowing treatment (between cold storage and sowing). Even so, levels introduced into both fumigated soil and containers were minimal. Generally, *Fusarium* seed infection below 10 percent does not result in extensive disease (James 1987b, 1987c, 1989).

All damped-off seedlings, ungerminated seed, and discarded seedcoats sampled from containers were colonized with *Fusarium*. Sporodochia, common on seeds and seedcoats (James 1992) (figure 3), were produced by several different *Fusarium* spp., the most common being *F. avenaceum* (Fr.) Sacc., *F. culmorum*, *F. solani*, and *F. sporotrichioides* Sherb. Since some of these species were also isolated from pre-sown seed, we suspect limited *Fusarium* introductions occurred. However, some fusaria were probably either already present within greenhouses or introduced on contaminated containers and/or media. Reused containers can be an important source of *Fusarium* contamination (Dumroese and others 1993; James and others 1988). However, the nursery routinely sterilizes containers with hot water between crops. Commercially prepared container media is usually pathogen-free (James 1985), although occasional batches may contain potentially pathogenic fungi (James and Gilligan 1984).

Our data indicated that much of the poor performance of white pine seed in both bareroot and container operations could not be attributed to disease-associated organisms. Although all seedlings with disease symptoms yielded *Fusarium* spp., these organisms were probably not responsible for poor germination because *Fusarium* levels found on seeds prior to and at sowing were much too low to account for the extensive germination problems. Most *Fusarium* inoculum probably came from the soil in bareroot seedlings; in container seedlings, several inoculum sources were probably involved.

Larsen (1922) called white pine seed "rebellious" because of its habitually delayed germination. This pattern of delayed germination is common in native stands. White pine seed may lay dormant in duff for up to six years (Hofmann 1917); Haig (1933) found less than one percent of seed stored in duff germinated during the first season, while most germinated the second year. Delayed germination may contribute to increased disease because emerging seedlings are disease-susceptible longer (James 1983, 1989, 1990). Germination is strongly influ-

enced by source family, indicating high heritability (Hoff 1986, 1987). Seedling production is often hampered by low germination capacity and energy even though the seed may have high viability (Dumroese and Wenny 1987). Therefore, white pine seed often has problems with uniform germination in many nurseries, including the Coeur d'Alene Nursery (James 1987a, 1987c, 1989). Seed dormancy is involved (Hoff 1987; Pitel and Wang 1985; Wang 1988). White pine seeds have dormancy sites in the seedcoat, papery membrane between the seedcoat and gametophyte, and the gametophyte (Hoff 1987). Usually, dormancy can be overcome by thoroughly soaking seeds (Partridge and others 1985; Pitel and Wang 1985), cold-moist stratification for 90 days (Krugman and Jenkinson 1974); Hoff 1987) which is much longer than for other conifer species (Hoff 1986; Kolotelo 1995; Schwandt 1983), and treating seeds with hormones (Pitel and Wang 1985). Often, white pine seed germinate better when given a warm-moist stratification treatment prior to cold-moist stratification (Anderson and Wilson 1966). In bareroot nurseries, fall sowing has long been the standard recommendation to overcome dormancy (Wahlenberg 1924a, 1924b, 1924c; Hoff 1978).

Table 1. Populations of *Fusarium*, *Trichoderma*, and *Pythium* in field 9 soil - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

	cfu/g[2]			
Transect[1]	Fusarium	Trichoderma	T/F Ratio[3]	Pythium
1	579	706	1.22	160
2	777	454	0.58	64
3	48	338	7.04	40
4	27	525	19.44	51
5	41	117	35.04	100
6	27	102	3.77	69
Average[4]	250	373	1.49	81
7	103	131	1.27	82
8	27	0	0	1
9	47	0	0	2
10	34	0	0	2
Average[5]	53	33	0.62	22
Overall Average	171	237	1.39	57

[1] Each transect consisted of 10 soil samples collected in an east to west pattern at 20-feet (3.1-m) intervals. Transect 1 began at the southern most portion of field 9.

[2] Colony-forming units per g of oven-dried soil.

[3] Ratio of *Trichoderma* to *Fusarium* populations.

[4] Transects 1-6 were within the portion of the field that had an incorporated corn cover crop the year prior to sowing.

[5] Transects 7-10 were within the portion of the field that was fallow the year prior to sowing.

Table 2. *Fusarium* isolations from diseased bareroot 1-0 western white pine seedlings and soil in field 9 - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

	Seedling Isolations[2]		Soil Isolations
Fusarium Species[1]	Percent Seedlings	Percent Colonization[3]	Percent of Isolates[4]
FOXY	88.2	77.8	93.2
FACU	0	0	4.4
FSOL	0	0	1.6
FEQU	0	0	0.8
All Species	88.2	77.8	100.0

[1] FOXY = *F. oxysporum*; FACU = *F. acuminatum*; FSOL = *F. solani*; FEQU = *F. equiseti*.

[2] 76 seedlings sampled.

[3] Percent of root pieces (2-4 sampled per seedling) infected with designated fungus.

[4] 249 total isolates evaluated.

An easy test to determine viability of western white pine seed is the excised embryo test (Johnson 1986). Healthy embryos will grow within a few days. Often, white pine seed stratified for 90-120 days still fail to germinate, often because of papery membrane-induced dormancy. An easy way to check if the problem is dormancy related is to clip the seedcoat (Hoff and Steinhoff 1986; Hoff 1987). Healthy seed with membrane dormancy will still germinate promptly.

Nursery growers require that seedling crops be established quickly and uniformly for cost-effective production. When seed problems occur, seedling losses can be very high and production goals unattained. Potentially pathogenic fungi, especially *Fusarium* spp., were present but not likely related to any significant level with the poor performance of this high-value, rust-resistant seed.

Table 3. *Fusarium* isolations from western white pine seeds, container-grown damped-off seedlings and ungerminated seed/discarded seedcoats - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

	Percent Infection				
	Seed[2]				
Fusarium Species[1]	From Cones	Cold Storage	At Sowing	Damped-off Seedlings[3]	Ungerminated Seed/ Discarded Seedcoats[4]
FCUL	0	0	4.6	32.5	23.7
FAVE	2.0	0	0.5	82.5	72.9
FACU	0	0	0.4	0	0
FSOL	0	0	0	5.0	13.6
FSPO	0	0	0	0	11.9
FOXY	0	0.2	0.5	0	0
FSAM	0.5	0	0.3	0	0
FPRO	0	0.8	0	0	0
FTRI	0	0.2	0	0	0
All Species	2.5	1.2	6.3	100.0	100.0

[1] FCUL = *F. culmorum*; FAVE = *F. avenaceum*; FACU = *F. acuminatum*; FSOL = *F. solani*; FSPO = *F. sporotrichioides*; FOXY = *F. oxysporum*; FSAM = *F. sambucinum*; FPRO = *F. proliferatum*; FTRI = *F. tricinctum*.

[2] Samples from cones and cold storage were 400 seeds; those taken at time of sowing were 800 seeds.

[3] 40 seedlings sampled; several were colonized with more than one *Fusarium* species.

[4] 59 ungerminated seed or discarded seedcoats sampled; several were colonized with more than one *Fusarium* species.

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