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ROOT DISEASES OF CONTAINER-GROWN PONDEROSA PINE SEEDLINGS USDA FOREST SERVICE BESSEY NURSERY, NEBRASKA

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ABSTRACT

Root disease of container-grown ponderosa pine seedlings at the USDA Forest Service Bessey Nursery was caused primarily by the pathogenic fungus *Fusarium proliferatum*. Other *Fusarium* spp. associated with roots of diseased seedlings and peat-based container growing media included *F. oxysporum*, *F. solani*, and *F. acuminatum*. One seedlot from the Grand Mesa-Umcompahgre National Forests was contaminated with very high levels of *F. acuminatum*. However, *F. proliferatum*, the likely major pathogen, was isolated only at very low levels from ponderosa pine seeds. Container growing media supported very high populations of *F. proliferatum* and a few other *Fusarium* spp. Reducing future losses will require preventing buildup of pathogen inoculum by implementing greenhouse, container, and seed sanitation accompanied by close monitoring of crops and removal of diseased seedlings during the growing season.

INTRODUCTION

Root diseases often adversely affect production of container-grown conifer seedlings in forest nurseries (Sutherland et al. 1989). Although several different groups of pathogens may be involved, *Fusarium* spp. are invariably the most commonly associated organisms. These fungi can enter container growing facilities in a variety of ways. They often contaminate conifer seeds (James 1986, 1987b), organic debris left from previous seedling crops (James et al. 1990, 1991), containers that are reused for several seedling crops (James et al. 1988), and may colonize soilless media within which seedlings are grown (James 1985). The most effective disease control efforts involve preventing

infection of susceptible seedlings by reducing levels of pathogen inoculum in and around greenhouses.

Several container-grown ponderosa pine (*Pinus ponderosa* Laws.) seedlings at the USDA Forest Service Bessey Nursery, Nebraska grown during 2004 exhibited root disease symptoms. These symptoms included foliar chlorosis and necrosis, stem and root decay, and seedling mortality. Within the container cells of several diseased seedlings, fungal sporulation (sporodochia) was evident on surface grit used to cover sown seed. Some seedlots had more diseased seedlings than others.

An evaluation was conducted to determine presence and level of potentially-pathogenic fungi on diseased seedlings, representative seeds of affected seedlots, and growing media surrounding roots of diseased seedlings.

MATERIALS AND METHODS

Eight seedlings displaying various levels of root disease symptoms from three seedlots were assayed for presence of potentially-pathogenic fungi on their roots. Some seedlings had mostly green foliage with necrosis restricted to needle bases or tips, whereas others displayed extensive foliar chlorosis and necrosis. Root plugs from sampled seedlings were carefully extracted from containers. Materials within plugs were divided into three portions: roots still attached to the seedling, roots that had become detached from the seedling (usually extensively decayed), and the surrounding peat-based growing media. Attached and detached roots were washed thoroughly under running tap water to remove adhering pieces of growing media. They were dissected into pieces about 5 mm in length. Fifteen root pieces from both attached and detached roots were randomly selected, surface sterilized in 0.525% aqueous sodium hypochlorite (10% standard household bleach), rinsed in sterile, distilled water, and placed on a selective agar medium for *Fusarium* and closely-related fungi (Komada 1975). Plates were incubated under diurnal cycles of cool, fluorescent light at about 24°C for 7-10 days. Selected isolates were transferred to carnation leaf agar (Fisher et al. 1982) and potato dextrose agar for identification using the taxonomy of Nelson et al. 1983. Percentages of sampled root pieces per seedling colonized by particular fungi were calculated.

Growing media adjacent to roots of each sampled seedling was assayed for populations of potential pathogens using a modified soil dilution technique. A subsample of each media sample was used to determine oven-dry weight in order to standardize all populations on a dry weight basis. This subsample was dried in an oven at about 100°C for 24 hrs. and the oven-dry weight factor calculated (wet weight divided by oven-dry weight). From each sample, 1.25 g of media were mixed with 200 ml of 0.3% water agar; one ml of the solution was placed on each of three plates of selective agar medium (Komada 1975). Plates were incubated and selected isolates identified as described above. Populations were expressed as colony-forming units per g (cfu/g) of oven-dry media.

Bulk-stored seeds from each of the three seedlots were assayed for presence of

potential pathogens. Fifty randomly-selected seeds per seedlot were placed directly on the selective agar medium. Plates were incubated and selected fungi identified as described above. Percent of sampled seed colonized by particular fungi was calculated.

RESULTS

An average of more than 90% of attached roots from diseased ponderosa pine seedlings were colonized by *Fusarium* spp. (table 1). In general, somewhat lower levels of *Fusarium* colonization was detected on plug roots that had become detached from diseased seedlings (table 2). Roots of most sampled seedlings were extensively colonized by these fungi, regardless of their level of disease symptom expression. There were no great differences in levels of root colonization among diseased seedlings from the three sampled seedlots. By far the most common *Fusarium* species colonizing roots was *F. proliferatum* (Matsushima) Nirenberg. The other two associated species were *F. oxysporum* Schlecht. and *F. solani* (Mart.) Appel & Wollenw.

Table 1. Colonization of attached roots of diseased container-grown ponderosa pine seedling with *Fusarium* species - USDA Forest Service Bessey Nursery, Nebraska

Seedling Number	Percent Root Colonization by *Fusarium* Species[1]			
	F. proliferatum	*F. oxysporum*	*F. solani*	All *Fusarium*
A1	100	0	0	100
A2	86.7	0	0	86.7
Average –A[2]	93.3	0	0	93.3
B1	100	0	0	100
B2	80	0	0	80
B3	93.3	0	0	0
Average – B[3]	91.1	0	0	91.1
C1	93.3	6.7	0	100
C2	53.3	0	26.7	80
C3	100	0	0	100
Average – C[4]	82.2	2.2	8.9	93.3
Average –All	88.3	0.8	3.3	92.5

[1] Based on 15 root pieces assayed from each seedling.
[2] Seedlot PP1249408303 [Pike-San Isabel National Forests]
[3] Seedlot PP1249408503 [Pike-San Isabel National Forests]
[4] Seedlot PP0424708203 [Grand Mesa-Umcompahgre National Forests]

Table 2. Colonization of detached roots of diseased container-grown ponderosa pine seedling with *Fusarium* species - USDA Forest Service Bessey Nursery, Nebraska

Seedling Number	Percent Root Colonization by *Fusarium* Species[1]			
	F. proliferatum	*F. oxysporum*	*F. solani*	All *Fusarium*
A1	93.3	0	0	93.3
A2	40.0	6.7	0	46.7
Average –A[2]	66.7	3.3	0	70.0
B1	86.7	13.3	13.3	100
B2	33.3	0	0	33.3
B3	40.0	20.0	13.3	73.3
Average – B[3]	53.3	11.1	8.9	73.3
C1	86.6	0	0	86.6
C2	60.0	0	0	60.0
C3	53.3	0	0	53.3
Average – C[4]	66.7	0	0	66.7
Average –All	61.1	5.0	3.3	70.0

[1] Based on 15 root pieces assayed from each seedling.
[2] Seedlot PP1249408303 [Pike-San Isabel National Forests]
[3] Seedlot PP1249408503 [Pike-San Isabel National Forests]
[4] Seedlot PP0424708203 [Grand Mesa-Umcompahgre National Forests]

Extremely high populations of *Fusarium* were detected within growing media adjacent to roots of diseased seedlings (table 3). Average populations for all samples exceeded 7000 cfu/g, several times the number considered potentially damaging (Hildebrand and Dinkel 1988; James et al. 1996). The major *Fusarium* species colonizing media was again *F. proliferatum*. Both other species isolated from roots (*F. oxysporum* and *F. solani*) also colonized growing media. In addition, a few isolates of *F. acuminatum* Ell & Ev. were encountered.

Even though high levels of *Fusarium* were isolated from seedling roots and adjacent growing media, levels contaminating pine seed, with one exception, were not very high (table 4). The exception was seed from the Grand Mesa-Umcompahgre National Forests (PP0424708203), which had high *Fusarium* contamination. The most commonly encountered *Fusarium* species on seeds was *F. acuminatum* (table 5). Two other species (*F. equiseti* (Corda) Sacc. and *F. proliferatum*) were isolated much less frequently. *Penicillium* spp. frequently contaminated seedcoats of all three pine seedlots (table 4); two other fungal genera (*Trichoderma* and *Phoma*) were isolated infrequently.

Table 3. Colonization of container growing media adjacent to diseased container-grown ponderosa pine seedlings with *Fusarium* species - USDA Forest Service Bessey Nursery, Nebraska.

Seedling Number[1]	Media *Fusarium* Populations [cfu/g][2]
A1	11733
A2	1973
Average – Lot A[3]	6853
B1	11840
B2	10933
B3	6720
Average – Lot B[4]	9724
C1	9553
C2	5600
C3	3200
Average – Lot C[5]	6151
Average – All Lots	7366

[1] Assayed media was adjacent to roots within containers.
[2] Four *Fusarium* species were isolated from media: *F. proliferatum* [956 isolates – 83.1%], *F. oxysporum* [125 isolates – 10.9%], *F. solani* [64 isolates – 5.6%], *F. acuminatum* [5 isolates – 0.4%].
[3] Seedlot PP1249408303 [Pike-San Isabel National Forests]
[4] Seedlot PP1249408503 [Pike-San Isabel National Forests]
[5] Seedlot PP0424708203 [Grand Mesa-Umcompahgre National Forests]

Table 4. Fungal contamination of three ponderosa pine seedlots from the Rocky Mountain Region - USDA Forest Service Bessey Nursery, Nebraska.

Seedlot	Percent Colonization[1]			
	Fusarium	*Penicillium*	*Trichoderma*	*Phoma*
A[2]	12	92	2	2
B[3]	10	60	0	0
C[4]	86	100	2	0
All Lots	36	84	1.3	0.7

[1] Based on a sample of 50 seeds/seedlot.
[2] Seedlot PP1249408303 [Pike-San Isabel National Forests]
[3] Seedlot PP1249408503 [Pike-San Isabel National Forests]
[4] Seedlot PP0424708203 [Grand Mesa-Umcompahgre National Forests]

Table 5. *Fusarium* species contaminating three ponderosa pine seedlots from the Rocky Mountain Region – USDA Forest Service Bessey Nursery, Nebraska.

Seedlot	*Fusarium* Species[1]			
	F. proliferatum	*F. acuminatum*	*F. equiseti*	All *Fusarium*
A[2]	2	10	0	12
B[3]	2	6	2	10
C[4]	0	74	12	86
All Lots	0.1	30.0	4.7	36.0

[1] Values in table are percent of sampled seeds contaminated with the particular *Fusarium* species based on a sample of 50 seeds/seedlot.
[2] Seedlot PP1249408303 [Pike-San Isabel National Forests]
[3] Seedlot PP1249408503 [Pike-San Isabel National Forests]
[4] Seedlot PP0424708203 [Grand Mesa-Umcompahgre National Forests]

DISCUSSION

Root diseases of container-grown ponderosa pine seedlings at the Bessey Nursery were caused primarily by *Fusarium proliferatum*, a species known to cause problems in greenhouses (Dumroese et al. 1993; James 1997, 2004; James et al. 1995, 1997). This species is not commonly seedborne (James 1997; James et al. 1997), as confirmed in this evaluation. Inoculum of *F. proliferatum* can be introduced into greenhouses via reused containers (James et al. 1988) and organic debris (James et al. 1990). Once present, *F. proliferatum* spreads rapidly because it readily forms chains of microconidia. These spores can become airborne (James et al. 1987; Nelson et al. 1983) and move within greenhouses from air blown via circulating fans. Near the end of the crop growing cycle, many roots of container conifer seedlings are often extensively colonized by *F. proliferatum* (James 1997; James et al. 1987). This occurs even though seedlings may not display above-ground disease symptoms (James and Gilligan 1988). The fungus is a good saprophyte that can extensively colonize organic matter including peat-based growing media (James 1997; Nelson et al. 1983). Tests to evaluate virulence of *F. proliferatum* isolates from container conifer seedlings indicated that most root-colonizing isolates have the potential to elicit disease (James et al. 1997). Therefore, seedling stress may play an important role in determining which plants become diseased as a result of root infection. Fortunately, when seedlings with roots colonized by *F. proliferatum* are out-planted on normal forest sites, these fungi usually do not elicit disease and are routinely replaced by other fungi, particularly mycorrhizal symbionts (Dumroese et al. 1993).

For two of the three treated seedlots, ponderosa pine seed contamination with *Fusarium* spp. was not extensive, even though root disease was evident. The most commonly-isolated seed contaminant was *F. acuminatum*, a species commonly encountered in forest

nurseries (James et al. 1991), but one that is not usually an aggressive pathogen (James 2000). Since this species was not isolated from roots of diseased seedlings and only infrequently from contaminated growing media, its importance in disease etiology is questionable.

It is unlikely that the growing media used at the nursery was colonized by *Fusarium* at the time of sowing. Pre-mixed, commercial, peat-based media is usually pathogen-free, and is often steam treated to reduce chances of pathogen contamination (James 1985). However, if pathogens, such as *F. proliferatum*, are introduced, the media is conducive to pathogen population increases (James 1985; James et al. 1990). This conduciveness may allow rapid pathogen spread and extensive root infection.

Root diseases of container conifer seedlings are best controlled by prevention rather than treatment following onset of disease symptoms (James et al. 1990, 1991). Reducing inoculum levels of potential pathogens on seeds, containers, within greenhouses and contaminated growing media may all be important. Sanitizing greenhouse interiors between seedling crops is especially important in reducing pathogen inoculum levels (James et al. 1990). Standard surface sterilants such as bleach or hydrogen peroxide may be effective. However, reducing levels of residual organic matter within and adjacent to greenhouses is also important since this material may often harbor pathogens. Another important preventive measure is to remove diseased seedlings periodically within greenhouses to reduce secondary spread (James et al. 1990). These seedlings should be removed completely from seedling growing areas and disposed of properly.

Seed treatments to reduce seedcoat contamination by potential pathogens should become routine, particularly for seedlots displaying germination or subsequent disease problems (Dumroese et al. 1988; James 1986, 1987a, 1987b). It may be important to screen certain seedlots suspected of carrying high pathogen populations (James et al. 1990). Level of pathogen contamination of tested seedlots can be quickly determined. Highly-contaminated lots should be treated with chemical sterilants, such as bleach or hydrogen peroxide (Dumroese et al. 1988; James et al. 1990; Wenny and Dumroese 1987). All seeds should be subjected to running water rinses for at least 48 hours either before or after stratification (James 1987a).

If the preventive procedures outlined above are implemented, future root disease losses should be minimized. Fungicides may effectively reduce levels of pre- and post-emergence damping-off, but are often not very effective in reducing root disease on older seedlings (James et al. 1987, 1990). Therefore, monitoring and inoculum reduction are important techniques in reducing disease losses.

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