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FUSARIUM BLIGHT OF CONTAINER-GROWN PONDEROSA PINE SEEDLINGS – MONTANA STATE NURSERY, MISSOULA, MONTANA

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ABSTRACT

Fusarium spp. were commonly associated with needle and stem blight of container-grown ponderosa pine seedlings at the Montana State Nursery, Missoula. The most common species isolated from diseased fascicle sheaths and stem lesions were *F. avenaceum* and *F. sporotrichioides*. *Fusarium proliferatum* was commonly isolated from healthy-appearing seedling roots, two lots of ponderosa pine seeds, media within which diseased seedlings were growing and detached seedcoats. Several other *Fusarium* species were also isolated infrequently from these substrates. It was suspected that *F. proliferatum* infected seedling roots from contaminated seedcoats shortly after germination and extensively colonized seedling roots and adjacent growing media. It was likely that *F. proliferatum* colonized seedling stem and twig tissues as well, acting as a non-pathogenic fungal endophyte. *Fusarium avenaceum* and *F. sporotrichioides* found on seeds may have contributed to subsequent above-ground disease. Extensive root colonization by *F. proliferatum* may have predisposed seedlings to top infection by the other two *Fusarium* spp. Although *F. avenaceum* and *F. sporotrichioides* are often associated with conifer seedlings, they are usually not considered very aggressive pathogens. However, both species were causing serious ponderosa pine seedling blight that spread rapidly under high-humidity greenhouse conditions. Management implications for reducing future losses are discussed.

INTRODUCTION

During the summer of 2002, scattered container-grown ponderosa pine (*Pinus ponderosa* Laws.) seedlings at the Montana State Nursery in Missoula displayed top blight symptoms. Affected seedlings had individual necrotic fascicle sheaths (figure 1). Necrosis often extended through the sheaths into the main stem, causing definitive lesions. Epiphytic fungal growth and pronounced fungal sporulation (sporodochia) were often evident at the base of affected needles near fascicle sheaths (figures 2 and 3). In some cases, terminal stem dieback was evident (figure 4) with the only non-necrotic tissues located at the base of affected seedlings. In extreme cases, entire seedlings were killed. The disease was fairly widespread, with some seedlots more affected than others. The disease spread rapidly and there was evidence of secondary disease spread.

Growers wanted information on the cause(s) of the problem as well as possible sources of pathogens and procedures for reducing further damage. Therefore, a series of isolations were made from diseased seedlings, discarded seedcoats and seeds of affected lots, and container growing media from several affected seedlings; associated micro-organisms were identified.

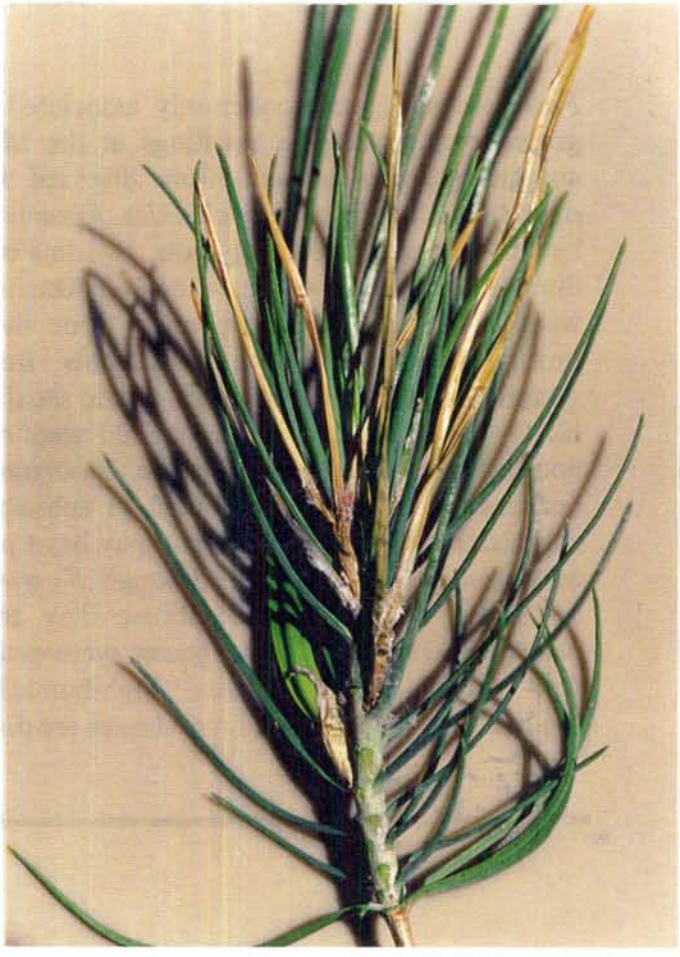

Figure 1. Top blight of container-grown ponderosa pine seedling – Montana State Nursery, Missoula. Individual groups of needles attached by fascicle sheaths were necrotic.

Figure 2. Close-up of blighted container-grown ponderosa pine needles from the Montana State Nursery, Missoula. The reddish-pink color indicate *Fusarium* sporodochia at the base of infected needles (arrow).

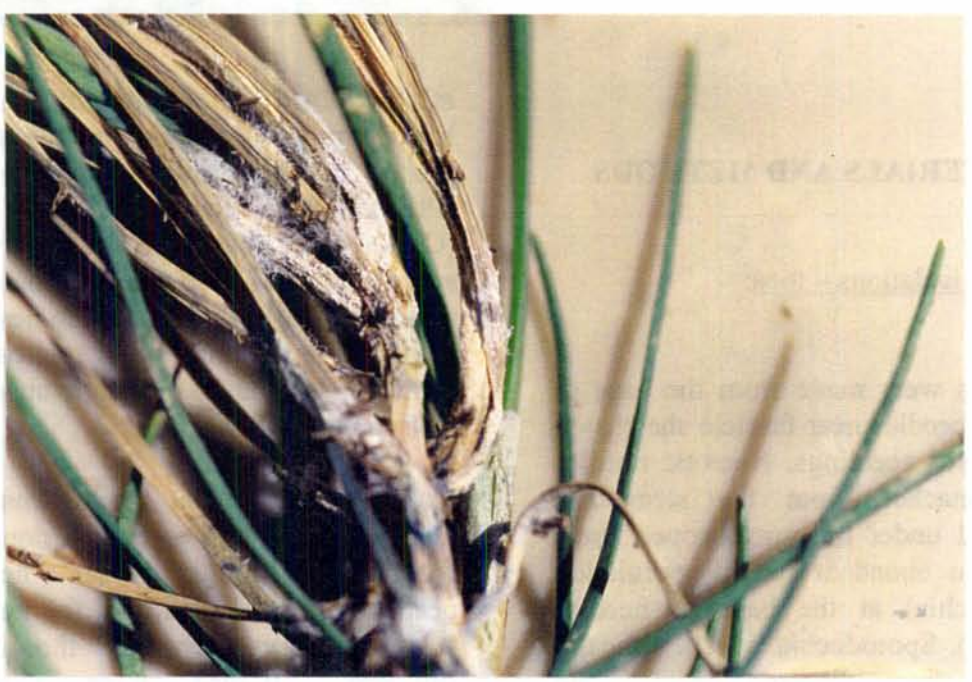

Figure 3. Extensive epiphytic fungal growth associated with blight of container-grown ponderosa pine seedlings – Montana State Nursery, Missoula. Necrosis extended from needles through fascicle sheaths and into the main stem causing lesions.

Figure 4. Top dieback of container-grown ponderosa pine seedlings – Montana State Nursery, Missoula. Necrosis spread from the top and seedlings down the main stem so that the only non-necrotic portion of seedlings was near the base.

MATERIALS AND METHODS

Seedling isolations – tops:

Isolations were made from the base of necrotic needles near fascicle sheaths on 14 diseased seedlings. Necrotic needles were detached from the stem and examined under the microscope. There was often abundant fungal sporulation (sporodochia) at the base of needles (figure 2). Sporodochia were aseptically removed from diseased needles and placed in sterile, distilled water. The resulting spore solution was decanted onto 2% water agar (WA) and incubated at about 24°C in the dark for about 18 hrs. Germinating spores were aseptically placed on both potato dextrose agar (PDA) and carnation leaf agar (CLA)(Fisher et al. 1982) to form single spore cultures. These were incubated for at least 10 days. Resulting colonies were identified using microscopic characteristics based on the taxonomy of Nelson et al. 1989.

Isolations were also made from the margins of main stem lesions on diseased seedlings. After necrotic needles had been detached, the necrotic stem area was washed thoroughly. Epidermal tissues were carefully removed with a sterile razor blade. Thin hand sections were aseptically extracted from the edge of necrotic lesions,

surface sterilized in a 10% bleach solution (0.525% aqueous sodium hypochlorite), rinsed in sterile, distilled water, blotted dry and placed on 2% WA. The sections were incubated for 48-96 hours at about 24°C in the dark. Emerging hyphae were transferred to PDA and CLA for fungal identification as described above. In general, isolations were made from 2-3 hand sections from each stem lesion.

Seedling isolations – roots:

Roots of diseased seedlings were sampled for colonization by potentially-pathogenic fungi. Seedlings were carefully extracted from containers and their roots washed thoroughly under running tap water. Roots that became detached from seedlings during washing were assayed separately from those still attached. Roots were cut into pieces 3-5 mm in length, surface sterilized, rinsed in sterile 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. Emerging fungi were transferred to PDA and CLA and identified as described above. Percentages of sampled root pieces colonized by different fungi were calculated.

Seed isolations:

Two types of isolations from seeds were conducted. The first was from two ponderosa pine seedlots (298 and 299) from bulk (cold) storage and the second was from detached seedcoats from seedlot 298. Sampled seeds were aseptically placed directly on Komada's medium, incubated as described above with selective isolates identified using standard single-spore procedures. Sample sizes were 228 for seedlot 298 and 231 for lot 299. Germination of sampled seeds on the agar medium was also monitored and summarized after 7 days' incubation. Forty-three detached seedcoats were collected throughout seedlot 298 in the greenhouse. Seedcoats were incubated directly on Komada's medium as described above and selected isolates were identified.

RESULTS

Several *Fusarium* spp. were consistently associated with blight of container-grown ponderosa pine seedlings. Three species (*F. avenaceum* (Fr.) Sacc., *F. sporotrichioides* Sherb. and *F. proliferatum* (Matsushima) Nirenberg) were associated with infected fascicle sheaths and stem lesions (table 1). The most common species on fascicle sheaths was *F. avenaceum*. However, the most common colonizer of both detached and attached seedling roots was *F. proliferatum* (table 2). This species was isolated from all sampled root pieces and occurred at very high population levels (> 7000 cfu/g) within the seedling growing media. *F. avenaceum* was detected at low levels on detached roots and within the growing media and *F. sporotrichioides* was not detected on seedling roots, but was found at low levels in growing media (table 2).

The major colonizer of sampled ponderosa pine seeds was *F. proliferatum*; this species was isolated from nearly all sampled seeds from both seedlots and from most of the detached

seedcoats samples (table 3). However, there were several other *Fusarium* species detected on the two seedlots as well. These included *F. sporotrichioides*, *F. avenaceum*, *F. acuminatum* Ell. & Ev. and *F. equiseti* (Corda) Sacc. All these species were isolated at relatively low levels, especially when compared to colonization by *F. proliferatum*. Germination of the two seedlots on agar medium were 71% and 57% for seedlots 298 and 299, respectively. However, since seeds were not incubated under optimum conditions, these germination rates probably are less than would be expected under ideal conditions.

Table 1. Colonization of ponderosa pine needle fascicle sheaths and stem lesions with *Fusarium* spp. – Montana State Nursery, Missoula.

Fusarium Species	Fascicle Sheaths[1]	Stem Lesions[1]	Stem Lesions[2]
F. avenaceum	57.1	42.9	33.3
F. sporotrichioides	35.7	42.9	46.7
F. proliferatum	7.1	42.9	33.3
All Species	100	50	100

[1] Percentage of seedlings sampled (14) infected with appropriate fungus. Stem lesions were found in 7 of 14 seedlings, all of which were colonized by *Fusarium* spp.
[2] Percentage of lesion margin pieces sampled (2-3 per seedling) colonized with appropriate fungus.

Table 2. Colonization of ponderosa pine seedling roots and soilless media with *Fusarium* and other fungi – Montana State Nursery, Missoula.

Isolation Location[1]	Fungus				
	Fusarium proliferatum	*Fusarium sporotrichioides*	*Fusarium avenaceum*	All *Fusarium*	*Trichoderma*
Roots-A[2]	100	0	0	100	1.1
Roots-D[2]	100	0	2.4	100	0
Media[3]	97.0	2.6	0.4	7013	0

[1] Roots – A = roots still attached to seedling; Roots – D = roots detached from seedlings during washing; Media = soilless (peat vermiculite) growing media.
[2] Percent of root pieces (275 sampled for attached and 125 sampled for detached roots) colonized by appropriate fungus.
[3] Under "All *Fusarium*" and "*Trichoderma*" the values are colony-forming units per gram of media; the values under the appropriate *Fusarium* species are the percent of isolates for that particular species.

Table 3. Colonization of stored ponderosa pine seed and detached seedcoats by *Fusarium* and other fungi – Montana State Nursery, Missoula.

Fungus	Seed Sample[1]		
	Lot 298	Lot 299	Detached – Lot 298
F. proliferatum	95.2	98.3	97.7
F. sporotrichioides	0.9	1.3	0
F. avenaceum	2.2	0	0
F. acuminatum	2.2	3.9	0
F. equiseti	0.9	0	0
Fusarium spp.[2]	0	0.4	0
All *Fusarium*	98.7	99.6	97.7
Penicillium	93.0	72.7	0
Trichoderma	1.3	1.3	0
Germination Percent[3]	71.0	56.7	--

[1] Values in table are percent of sampled seed (228 for lot 298; 231 for lot 299; 43 for detached seedcoats) colonized by appropriate fungus.
[2] Unidentified species within section *Liseola*.
[3] Germination percent of sampled seed after incubation for 7 days on agar medium.

DISCUSSION

Top blight of container-grown ponderosa pine seedlings was caused by a combination of three different *Fusarium* species: *F. avenaceum*, *F. sporotrichioides*, and *F. proliferatum*. All three species were isolated from diseased fascicle sheaths and/or stem lesions on blighted seedlings. In addition, *F. proliferatum* extensively colonized roots of blighted seedlings and seeds from two diseased seedlots.

Fusarium proliferatum may have systemically colonized diseased seedlings from colonized roots because it was frequently isolated from stem tissues. Its presence on non-diseased roots and stems would indicate that this species may sometimes act as a non-pathogenic endophyte. Although endophytic fungi may act to suppress disease within infected host plants (Bettucci and Alonso 1997; Bultman et al. 1998), it is also possible that extensive endophytic colonization by *F. proliferatum* may have stressed infected seedlings sufficiently to make them more susceptible to attack by the other two *Fusarium* species.

Fusarium proliferatum, which is a member of an important group of plant pathogenic fungi with the teleomorph *Gibberella fujikuroi* (Klittich and Leslie 1992; Kuhlman 1982; Leslie 1991, 1995; Nirenberg and O'Donnell 1998), has routinely been isolated from roots of

healthy and diseased container-grown conifer seedlings (Dumroese et al. 1993, 1995; James 1987a, 1987b, 1987c, 1987d, 1987f; James et al. 1989a, 1991; Shrimpton and Williams 1989). This fungal species may be pathogenic on conifers (Huang and Kuhlman 1990; James 1993; James et al. 1989b, 1995; Viljoen et al. 1995) as well as numerous other plant hosts (Abbas et al. 1988; Abdalla et al. 2000; Cotton and Munkvold 1998; Elmer 1990, 1991, 1995; Leslie et al. 1990; Marin et al. 1998; Ocumb and Kommedahl 1994; Wagih et al. 1989). It may readily colonize conifer seeds and spread during stratification (Fraedrich and Miller 1995; Hoefnagels and Linderman 1999). Pathogenicity of *F. proliferatum* isolates on different host plants may often vary widely (Elmer and Ferrandino 1992; Schreuder et al. 1995). Some of this variability may be related to its true taxonomic position within the *Fusarium* section *Liseola*. For example, earlier taxonomy of this section provided for four species (Nelson et al. 1983). However, recent use of molecular systematics (O'Donnell et al. 1998), indicated that there are at least 36 species within the section, several of which may have been formerly classified as "*F. proliferatum*". Therefore, this morphological species may be quite diverse and include pathogens, saprophytes and even potential biological control organisms (Bakshi et al 2001; Falk et al. 1995; Magie 1980). Its ability to form large numbers of microconidia in long chains as well as macroconidial sporodochia on stems of diseased seedlings facilitate spore dispersal in water and air currents (Fisher et al. 1983; James 1992; Kuhlman 1982; Nelson et al. 1990). The fungus rapidly spreads within greenhouses and probably within seed storage facilities. *Fusarium proliferatum* may also be spread within greenhouses by fungus gnats (James et al. 1994).

The fungal species most often isolated from blighted fascicle sheaths and sporulating readily at the base of infected needles was *F. avenaceum*. Although this species lacks microconidia and chlamydospores (Hargreaves and Fox 1978; Klotz et al. 1988; Nelson et al. 1983; Warren and Kommedahl 1973), it produces abundant macroconidia in orange-pink sporodochia (Jenkinson and Parry 1994a; Kiecana et al. 2002; Morgan 1983; Sangalang et al. 1995) often on above-ground plant parts (Bonish and Di Menna 1993; Sprague 1960). Spores are readily disseminated by water splash (Jenkinson and Parry 1994b). *Fusarium avenaceum* has often been isolated from conifer seedlings, particularly from their roots and seeds (Hoefnagels and Linderman 1999; James 1987e; James et al. 1989a). It has also been considered pathogenic on a number of different plants, including cereals (Duthie et al.1986; Kiecana et al. 2002; Kommedahl et al. 1975; Lin and Cook 1979; Oswand 1949; Price 1984; Sprague 1960), carnations (Laudon 1971), various nursery crops (Gurusiddaiah et al. 1979; Mwanza and Kellas 1987) and conifer seedlings (Asiegbu et al. 1999a, 1999b; Gordon 1959; James 1993; Kulakova 1964; Morgan 1983; Tint 1945a, 1945b) However, in most cases, it is believed to be either a weak pathogen (Asiegbu et al. 1999; Johnston and Greaney 1942; Parry and Pegg 1985; Stack and McMullen 1985) or nonpathogenic (Burgess et al. 1975; Rathbun-Gravatt 1931). Apparently, there is much variation among different isolates of *F.*

avenaceum (Forbes and Dickinson 1977; Sangalang et al. 1995) and some isolates are important producers of plant and animal toxins (Simpson et al. 2001).

Isolates of *F. sporotrichioides* were likewise frequently obtained from diseased fascicle sheaths and stem lesions. This species has sometimes been considered an aggressive pathogen of conifer seedlings (James 1985; Mittal and Wang 1993; Rathbun 1922; Rathbun-Gravatt 1925, 1931; Seemuller 1968; Singh and Mittal 1989; Tint 1945a). It is also a common colonizer of conifer seeds (Hoefnagels and Linderman 1999; James 1987e; Mittal and Wang 1987, 1989, 1993; Singh and Mittal 1989) and nursery soils (James 2002a, 2002b). Under controlled conditions, tests of several isolates from forest nurseries indicated a wide range of virulence on young conifer germinants (James and Perez 1999). Isolates of *F. sporotrichioides* have been associated with diseases of other plant hosts including water milfoil (Andrews and Hecht 1981) and various cereals (Chelkowski et al. 1989; Duthie et al. 1986). This fungus produces important toxins (trichothecenes) that are damaging to both plants and animals (Brown et al. 2001; Desjardins et al. 1989, 1993; Moss and Frank 1985). It readily produces sporodochia on the aerial portion of plants (Gordon 1959; James 1992; Vargo and Baumer 1986).

MANAGEMENT IMPLICATIONS

All of the *Fusarium* spp. associated with this disease may have acted synergistically to cause severe blight on container-grown ponderosa pine seedlings. The disease spread rapidly and caused important losses under the ideal conditions for spread and infection within greenhouses. Disease impacts can be reduced by applications of selected fungicides that are efficacious against *Fusarium* spp. (thiophanate methyl [Cleary's 3336®], azoxystrobin [Medallion®], proxystrobin [Heritage®]). Future losses can be reduced by limiting pathogen inoculum on seed, organic debris within greenhouses and nearby weeds, which can serve as pathogen reservoirs (Jenkinson and Parry 1994a). It is also important to monitor seedling crops to locate disease as soon as it becomes apparent so that control measures can be rapidly implemented.

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