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DISEASES OF CONTAINER-GROWN CONIFER AND BRUSH SEEDLINGS USDA FOREST SERVICE LUCKY PEAK NURSERY BOISE, IDAHO

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ABSTRACT

Potentially-pathogenic fungi associated with diseases of container-grown Douglas-fir, Engelmann spruce, ponderosa pine, sagebrush, and bitterbrush seedlings at the USDA Forest Service Lucky Peak Nursery were identified. Several species of *Fusarium*, particularly *F. proliferatum*, extensively colonized roots of all diseased seedlings except bitterbrush. Foliage of diseased bitterbrush seedlings was commonly colonized with *Botrytis cinerea*. Needle and top dieback was a common symptom of root diseased conifer seedlings. Root diseased sagebrush seedlings were commonly grouped within containers where extensive mortality occurred. Root diseases and *Botrytis* are best prevented by using disease-free seed, containers, and growing media. Organic matter harboring pathogen inoculum should be removed from greenhouses between crops. Periodic removal of seedlings with disease symptoms is important to reduce secondary pathogen spread. Chemical pesticides are more effective for controlling *Botrytis* than for root diseases caused by *Fusarium* spp. Pesticide usage should be regulated to ensure that pathogens do not develop resistance to chemicals.

INTRODUCTION

A new greenhouse facility was recently completed at the USDA Forest Service Lucky Peak Nursery, Boise, Idaho to grow a variety of conifer and brush seedlings in containers for reforestation and restoration purposes. Several different plant species are being grown within the greenhouse. During 2004, disease symptoms were noticed on Douglas-fir (*Pseudotsuga menziesii* Franco var. *glauca* [Mayr.] Sudw.), Engelmann spruce (*Picea engelmanni* Parry), ponderosa pine (*Pinus ponderosa* Laws.), sagebrush (*Artemesia tridentata* Nutt.), and bitterbrush (*Pushia tridentata* [Pursh] DC.) seedlings. All seedlings were being grown within new styrofoam containers. Symptoms on Douglas-fir included needle tip and top dieback, and crown mortality (figure 1). Affected Engelmann spruce displayed varying degrees of needle necrosis; severely-affected seedlings were entirely necrotic (figure 2). Disease symptoms on sage-brush were very prominent; relatively large groups of seedlings were killed and it appeared that seedlings had died quickly (figure 3). Limited foliage necrosis was evident on diseased bitter-brush and ponderosa pine seedlings. Many affected seedlings had extensive root decay within container plugs. Examples of diseased seedlings were analyzed for presence of potentially-pathogenic fungi.

MATERIALS AND METHODS

Varying numbers of conifer and brush seedlings with disease symptoms were sampled (table 1). Root systems of sampled seedlings were washed thoroughly under running tap water to remove particles of growing media. They were then dissected into pieces approximately 5 mm in length. Selected root pieces were surface sterilized with 0.525% aqueous sodium hypochlorite (10% household bleach), rinsed in sterile water, and placed on a selective agar medium for *Fusarium* and closely-related pathogenic fungi (Komada 1975). Ten or fifteen root pieces were placed on each agar plate. Plates were incubated under diurnal cycles of cool, fluorescent light at about 24°C for 7 days. Selected fungi emerging from root pieces were transferred to carnation leaf agar (Fisher et al. 1982) and potato dextrose agar (PDA) for identification using the taxonomy of Nelson et al. 1983. Percent of sampled root pieces colonized by particular *Fusarium* species was calculated.

Twenty-five Douglas-fir seedlings with disease symptoms from the greenhouse and ten from a nearby shadehouse were sampled for pathogens colonizing their stems to determine if seedlings had become systemically colonized. Three stem pieces (about 3 mm each) from each seedling were analyzed for *Fusarium* colonization using the same procedures for determining root colonization.

Above-ground necrotic foliage from each of the sampled brush species was washed thoroughly and incubated in moist chambers for 3-5 days at about 24°C to induce sporulation of associated fungi. Selected isolates of associated fungi were transferred to 2% water agar and then subsequently transferred to PDA for identification using micro-scopic examinations.

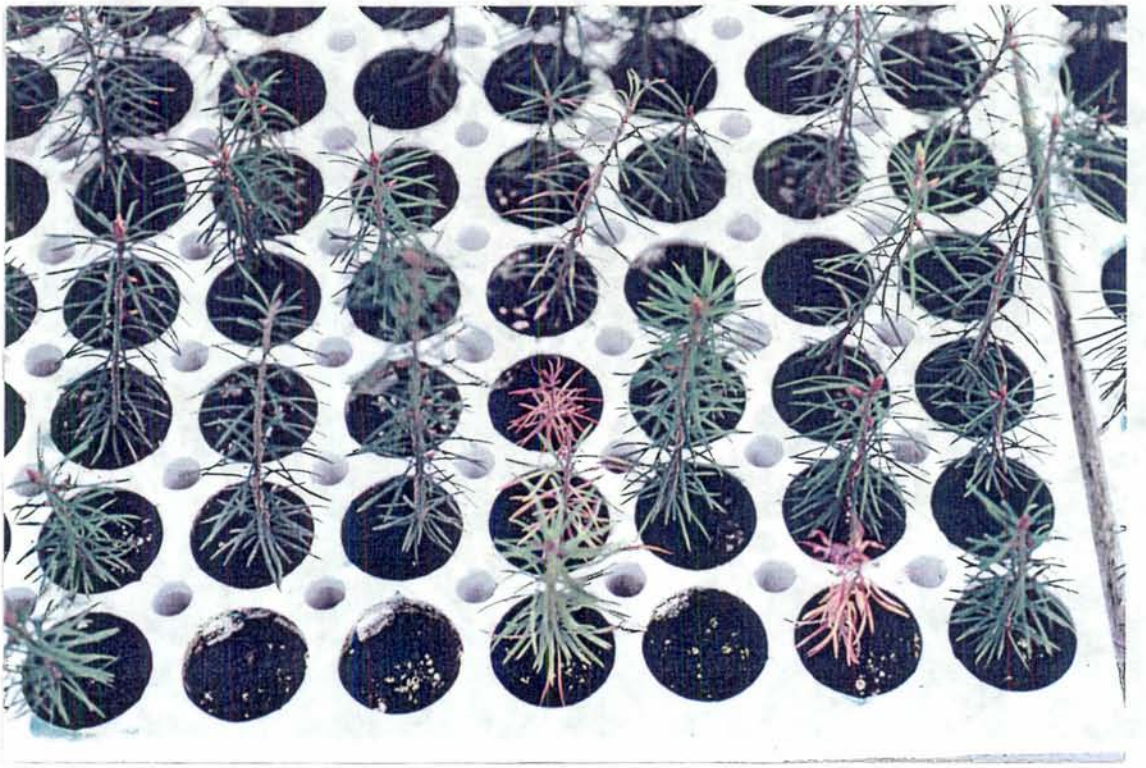

Figure 1. Root disease of container-grown Douglas-fir seedlings - USDA Forest Service Lucky Peak Nursery. Affected seedlings initially exhibited needle tip dieback and foliar chlorosis. At later stages, entire seedlings became necrotic.

Figure 2. Root disease of container-grown Engelmann spruce seedlings - USDA Forest Service Lucky Peak Nursery. Affected seedlings displayed needle necrosis throughout their crowns.

Figure 3. Root disease of container-grown sagebrush seedlings - USDA Forest Service Lucky Peak Nursery. Affected seedlings were usually located in groups of varying sizes, indicating secondary pathogen spread.

RESULTS

Root infection and colonization of diseased container-grown seedlings by various *Fusarium* spp. is summarized in table 1. With the exception of some bitterbrush seedlings, all sampled plants with disease symptoms were infected with *Fusarium* with levels of root colonization at or near 100%. Of a total of 1055 root pieces sampled from the three conifer species and sagebrush, 99.7% were colonized with one or more *Fusarium* spp. The most commonly-isolated *Fusarium* species was *F. proliferatum* (Matsushima) Nirenberg; this species colonized nearly 95% of sampled root pieces. Other *Fusarium* species isolated, although at much lower levels, included *F. oxysporum* Schlecht., *F. solani* (Mart.) Appel & Wollenw., *F. acuminatum* Ell. & Ev., and *F. sambucinum* Fuckel.

Stem colonization of diseased Douglas-fir seedlings by *Fusarium* spp. is summarized in table 2. Similar to the roots of diseased seedlings, most of the sampled stem pieces from either green-house or shadehouse collections were extensively colonized with *Fusarium*. The same *Fusarium* species isolated from seedling roots were also isolated from stems, indicating systemic spread of pathogens upward from roots. *Fusarium proliferatum* was again the most commonly isolated species. *Fusarium solani* was much more frequently isolated from stem tissues than from roots.

Table 1. Root colonization of diseased conifer and brush container-grown seedlings with *Fusarium* spp. - USDA Forest Service Lucky Peak Nursery, Boise, Idaho.

Species[1]	Sample No.[2]	Percent Inf[3]	Root[4] Pieces	Percent Root Pieces Colonized[5]					
				FPRO	FOXY	FSOL	FACU	FSAM	ALL
DF-1	25	100	250	92.4	15.2	4.0	0	0	99.6
DF-2	10	100	100	86.0	10.0	8.0	1.0	4.0	100.0
All DF	35	100	350	90.6	13.7	5.1	0.3	1.1	99.7
ES	15	100	225	99.1	27.1	0	0	0	100.0
PP	18	100	180	93.9	7.2	1.1	0	0	100.0
Conifers[6]	68	100	755	93.9	16.2	2.6	0.1	0.5	99.9
SB	20	100	300	96.0	0.3	1.0	0	0	99.3
All[7]	88	100	1055	94.5	11.7	2.2	0.1	0.4	99.7
BB	6	83.3	90	4.4	0	0	12.2	0	16.7

[1]Species: DF-1 = Douglas-fir from greenhouse; DF-2 = Douglas-fir from shadehouse [previous crop]; ES = Engelmann Spruce; PP = Ponderosa Pine; SB = Sagebrush; BB = Bitterbrush.
[2]Number of diseased seedlings sampled
[3]Percent of sampled seedlings infected with *Fusarium* spp.
[4]Number of root pieces sampled [10 or 15 per seedling]
[5]Percent of sampled root pieces colonized by appropriate *Fusarium* species; FPRO = *F. proliferatum;* FOXY = *F. oxysporum*; FSOL = *F. solani*; FACU = *F. acuminatum*; FSAM = *F. sambucinum*; All = All *Fusarium* species.
[6]All conifers combined
[7]All seedlings EXCEPT bitterbrush, which had very low *Fusarium* root infection.

Table 2. Colonization of stem pieces from diseased container-grown Douglas-fir seedlings by *Fusarium* spp. - USDA Forest Service Lucky Peak Nursery, Boise, Idaho.

Sample[1]	No. Pieces[2]	Percent Stem Piece Colonization[3]					
		FPRO	FOXY	FSOL	FACU	FSAM	ALL
DF-1	75	84.0	18.7	29.3	0	0	100.0
DF-2	30	60.0	6.7	30.0	6.7	10.0	83.3
ALL DF	105	77.1	15.2	29.5	1.9	2.9	95.2

[1]DF-1 = 25 diseased seedlings sampled from the greenhouse; DF-2 = 10 diseased seedlings sampled from the shadehouse.
[2]Number of stem pieces sampled [3 per seedling]
[3]Percent colonization of sampled stem pieces by appropriate *Fusarium* species; FPRO = *F. proliferatum*; FOXY = *F. oxysporum*; FSOL = *F. solani*; FACU = *F. acuminatum*; FSAM = *F. sambucinum*; All = All *Fusarium* species.

Five of the six bitterbrush seedlings sampled for foliar-colonizing pathogens were extensively colonized by *Botrytis cinerea* Pers. ex Fr.. This fungus was isolated most frequently from necrotic tissues, but was also found on adjacent green foliage. No *Botrytis* was found colonizing sagebrush foliage. Foliage of both diseased bitterbrush and sagebrush was extensively colonized by *Alternaria alternata* (Fr.) Keissler, *Penicillium* spp., and three species of *Phoma* [*P. eupyrena* Sacc., *P. glomerata* (Corda) Wollenw. & Hochapf. and *P. pomorum* Thum.]. All these fungi are suspected to be secondary saprophytic colonizes of tissues killed by *Botrytis*.

DISCUSSION

Container-grown seedlings grown within greenhouses are often very susceptible to fungal-caused diseases (James et al. 1993; Sutherland et al. 1989). Persistent periods of high humidity and temperatures that occur within greenhouses are optimum for growth, sporulation, spore germination, and plant infection by many common root and foliar pathogens (James et al. 1990; Sutherland et al. 1989). The two most damaging groups of pathogens are *Fusarium* spp. and *Botrytis cinerea*. Several *Fusarium* spp. commonly cause damping-off of young seedlings before and shortly after emergence and root disease of older seedlings (James et al. 1988b, 1988c, 1987, 1991). Previous investigations (James 1992, 1993; James et al. 1987) have indicated that *F. oxysporum* is often the most damaging damping-off species, primarily because it is introduced into greenhouses on contaminated seed (James 1986, 1987). As the growth cycle progresses, incidence of *F. oxysporum* usually declines and *F. proliferatum* tends to increase dramatically, often replacing *F. oxysporum* as the most important pathogen (James and Perez 1998; James et al. 1995). One factor that may responsible for rapid spread of *F. proliferatum* within greenhouses is ability of the fungus to form long chains of microconidia that are readily disseminated by air movements (James 1997; James et al. 1995; Nelson et al. 1983). Greenhouse fans or ambient air circulation resulting from opening sides of greenhouses probably increase rapid spread of this important pathogen. Roots are often infected with *F. proliferatum*, and sometimes other *Fusarium* spp., but seedlings may not express disease symptoms for some time (James 1997; James and Gilligan 1988; James et al. 1987, 1991). Tests have shown that most *F. proliferatum* isolates obtained from both diseased and healthy-appearing seedlings are capable of causing rapid, extensive disease, at least under ideal conditions for disease development (James 1989b, 1997). Therefore, it is suspected that stress plays an important role in the timing and intensity of disease symptom expression (James 2004b; James et al. 1990). When warm temperatures persist (Bloomberg 1985; James et al. 1991) and when seedlings are stressed to promote budset toward the end of the growing cycle (James et al. 1987, 1990), disease symptoms may quickly appear. Since most of the roots of diseased seedlings have usually been infected for a long time with *F. proliferatum* (James et al. 1987, 1995), appearance of symptoms indicates that the host-pathogen interaction has shifted in favor of the pathogen.

All of the other *Fusarium* spp. isolated from diseased seedling roots have frequently been encountered in greenhouse nurseries (James 2000, 2005b; James and Perez 2000; James et al. 1988a, 1989a, 1991). In most cases, these other species are not important pathogens, as revealed by controlled pathogenicity tests (James 2000; James and Perez 2000). The most likely exception is *F. oxysporum*. Some isolates of this species can be aggressive pathogens, whereas others are entirely saprophytic (Gordon and Martyn 1997; Gordon and Okamoto 1992: James et al. 2000). Both pathogenic and non-pathogenic isolates of *F. oxysporum* appear similar morphologically (Bloomberg and Lock 1972; Gordon and Martyn 1997; James et al. 1989a, 2000) so it is difficult to easily identify which isolates are potentially important pathogens. Fortunately, recent genetic analysis work has indicated that molecular markers for pathogenic isolates may exist (Stewart et al. 2004). If such markers are consistently associated with pathogenic isolates, techniques can be developed to rapidly screen populations of *F. oxysporum* for disease risk assessments.

It was interesting that *F. solani* was much more frequently isolated from the stems of diseased seedlings than their roots. This taxon, like *F. oxysporum*, probably comprises several individual species that behave differently on a wide range of plant hosts. For example, some isolates commonly cause root decay (Adams et al. 1968; Hartley 1918; Snyder et al. 1959). Others cause stem cankers (Kabir 1965), and still others are commonly isolated from plant tissues without inducing any disease response (endophytes)(Bloomberg 1966; James 2004a). Therefore, the role of the isolates of *F. solani* obtained from stems of diseased Douglas-fir seedlings in inciting disease is unknown. Most forest nursery isolates tested so far were not aggressive pathogens of Douglas-fir (James and Perez 2000).

Botrytis cinerea is a very common pathogen of container-grown seedlings in forest nurseries (James 1984, 1994; Jarvis 1980). It is especially virulent on some plant species, especially those that tend to form dense canopies near the end of their growth cycle. This non-specialized pathogen has a very wide host range (James 1984; Jarvis 1980; McCain and Smith 1978) and usually initiates infection on necrotic plant tissues, particularly within the base of the seedling canopy (James 1984; McCain and Smith 1978). When moisture persists on foliage for prolonged periods, *Botrytis* rapidly spreads and can cause extensive seedling damage in very short periods of time. This pathogen tends to cause most of its problems near the end of the growth cycle because of full seedling canopies and because cooler temperatures, which encourage pathogen development, are common in the late summer or fall (James 1984, 1994; Jarvis 1980).

Management of diseases of container-grown seedlings should emphasize prevention rather than trying to therapeutically reduce damage. It is very important that pathogen inoculum within seedling growing environments be reduced as much as possible. This requires using pathogen-free seed (James 1986, 1987) and clean containers, particularly styrofoam containers that are reused for several seedling crops (James et al. 1988d, 1990, 1991). Peat-based growing media are usually pathogen-free

(James 1985; James et al. 1990), although some exceptions have been found (James 2005a). Interior greenhouse environments should be devoid of organic matter that may harbor pathogens, particularly since most fungi capable of causing diseases are also very good saprophytes (James 1984; James et al. 1991; Maude 1980).

One of the most important ways to reduce disease impacts is to institute a program of frequent seedling observation. During these surveys, all diseased seedlings should be removed completely from the greenhouse environment (James et al. 1990). A conscientious survey and diseased-seedling-removal program will greatly reduce incidence of secondary disease spread.

Direct control measures to reduce impacts of Fusarium root disease and Botrytis blight involve application of fungicides. These materials may be biological control agents or chemical pesticides. Commerically-available biocontrol agents were developed for agricultural crops and not plant species usually grown in forest nurseries. This may be why they often have limited ability to adequately control target pests in forest nurseries (Dumroese et al 1996, 1998; Mousseaux et al. 1998). They primarily contain antagonistic bacteria or fungi that either compete with or attack pathogenic fungi (James 2002b; James et al. 1990). Probably the most promising agents evaluated so far against pathogenic *Fusarium* spp. are other *Fusarium* strains that are non-pathogenic and compete well with pathogens for host plant infection and colonization (James 2002).

Chemical pesticides should only be used if other control measures are ineffective (James 1988). Pesticides should be used sparingly to help preclude development of resistance by pathogen populations (Bollen and Scholten 1971; Gilman and James 1980; James 1984; James and Woo 1984; Maude 1980). It is best not to continuously use the same fungicide as this tends to place a lot of pressure on pathogen populations to mutate genetically (James 1984; Maude 1980). Some resulting mutations result in resistance to the applied chemical (Bollen and Scholten 1971; Gilman and James 1980; James and Gilligan 1985). However, if several different pesticides, particularly with different modes of action, are rotated, there is less pressure on pathogens to develop resistance (Bollen and Scholten 1971; Maude 1980). Most chemical pesticides are not effective therapeutically, i.e., they do not kill pathogens that have already infected plant tissues (Dumroese et al. 1990; James 1988). They are usually designed to help prevent fungal spore germination and subsequent infection of plants (James 1988; McCain and Smith 1978).

CONCLUSIONS

Some level of root and foliage disease is inevitable when susceptible plants are grown within greenhouses (Dumroese et al. 1993; James et al. 1990: Sutherland et al. 1989). All conifer species can be affected, although some seem less damaged than others. Douglas-fir and Engelmann spruce are often more damaged than other conifers such as ponderosa and lodgepole pine (James 1992, 1993; James et al. 1984, 1987, 1991), although exceptions have been

found. Apparently, sagebrush is very susceptible to Fusarium root disease, at least when grown under greenhouse conditions. Although not verified by experimental data, it is suspected that similar strains of *Fusarium* are capable of attacking all the different hosts that were affected at the Lucky Peak Nursery. If this is the case, pathogens are easily capable of spreading from one host species to another. Therefore, it is important to conduct timely inspections of stock throughout the growth cycle and remove and diseased seedlings when they are found. If conditions are optimal for pathogen development and spread and timely control measures are not instituted, epidemics of Fusarium root disease can occur and sometimes entire crops can be lost (James et al. 1984). Therefore, a proactive approach to managing greenhouse diseases is usually beneficial.

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