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**FUSARIUM SPECIES ASSOCIATED WITH POST-EMERGENCE DAMPING-OFF  
AND ROOT DISEASE OF YOUNG CONTAINER-GROWN DOUGLAS-FIR SEEDLINGS  
USDA FOREST SERVICE NURSERY, COEUR D'ALENE, IDAHO**

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**ABSTRACT**

*Fusarium* spp. associated with post-emergence damping-off of 1-week-old germinants and root disease of 2-month-old seedlings were identified from three seedlots at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. *Fusarium avenaceum*, *F. acuminatum*, and *F. equiseti* were most common on germinants; other *Fusarium* spp. (*F. sambucinum*, *F. oxysporum* and *F. proliferatum*) were isolated from root-diseased seedlings, but not young germinants. A succession of fusaria on the roots of container-grown Douglas-fir seedlings likely occurs throughout the growing season.

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**INTRODUCTION**

Production of container-grown seedlings has steadily increased during the past several years at the USDA Forest Service Nursery in Coeur d'Alene, Idaho. Several different conifer species are produced in containers, but one of the most important is inland Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco). Unfortunately, root diseases have often caused serious limitations to production of container-grown Douglas-fir at this and other nurseries in the inland Northwest (James 1984; James and others 1991). Past evaluations (James 1988; James and others 1987, 1988b) have shown that much associated root disease of this and other conifer species develops near the end of the growth cycle when seedlings may become stressed due to induced restrictions in watering and fertilizer applications to stop seedling growth and set buds. However, careful examination of seedling crops indicate that some root disease can usually be found on very young seedlings as well (James and other 1987). Likewise, post-emergence damping-off may be common on very young germinants, especially within certain seedlots (James 1986).

Several different *Fusarium* spp. have been associated with root diseases of container-grown Douglas-fir (James and others 1989). Some of these fungi are also readily detected on roots of seedlings without root disease symptoms (James and Gilligan 1988; James and others 1988b). It seems likely that some of these potential pathogenic fusaria are introduced into container seedling operations on conifer seed (James 1986, 1987). Others may enter on contaminated containers (James and others 1988a) or be present within the interior of greenhouses (James and others 1987), causing infection of seedlings early in the growth cycle. It is also likely that some fusaria are introduced and cause infection when seedlings become older. Therefore, there is likely a succession of fusaria that infect conifer seedling roots throughout the production phase in container nurseries. Some of these fungi may potentially cause more problems than others with respect to their ability to incite disease.

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## MATERIALS AND METHODS

To begin investigations into possible succession of fusaria in container-grown Douglas-fir seedlings, crops were sampled at the USDA Forest Service Nursery, Coeur d'Alene, Idaho, early in the growth cycle and populations of *Fusarium* spp. associated with seedlings exhibiting disease symptoms were ascertained. One sample was collected from one week-old germinants from the spring 1992 crop. Young germinants displaying typical post-emergence damping-off symptoms from three representative seedlots were collected for laboratory analysis. Germinants were washed carefully to remove pieces of growing media, surface sterilized for 30 seconds in a 10 percent bleach solution (0.525 percent aqueous sodium hypochlorite), rinsed with sterile water, and placed on a selective agar medium for *Fusarium* spp. (Komada 1975). Plates were incubated under diurnal cycles of cool, fluorescent light at about 24°C for 7-10 days. Representative *Fusarium* isolates were transferred using single spore techniques (Nelson and others 1983) to potato dextrose agar and carnation leaf agar (Fisher and others 1982) to facilitate identification.

Two months following the first sample, a second sample of root-diseased seedlings was collected from the same seedlots. Selected seedlings displayed various levels of foliar chlorosis or necrosis; needle tip dieback was often indicative of root disease (James and others 1987, 1991). Diseased seedlings were carefully removed from container cells with sterile forceps and placed in sterile water for transport to the laboratory. Root systems were carefully washed to remove particles of growing media, dissected into pieces approximately 5 mm in length, surface sterilized, and ten randomly-selected root pieces were incubated on the selective agar medium as described above.

Several taxonomic guides for the genus *Fusarium* (Booth 1971; Gerlach and Nirenberg 1982; Joffe 1974; Nelson and others 1983) were consulted for identification of associated isolates. Some of these guides were more difficult to work with because they were quite subjective. Experience has shown that the treatise by Nelson, Toussoun and Marasas (1983) provides very consistent results when identifying these organisms. Characters emphasized by this treatise seem to better consistently differentiate isolates than those described by other taxonomists. Because of extreme variability within the genus *Fusarium* (Nelson and others 1983), there is always a range of morphological characters describing an individual taxon. Within certain species, there is often much variability; however, certain conserved characteristics are available to identify individual species. Therefore, the treatise by Nelson, Toussoun, and Marasas (1983) was used to identify *Fusarium* spp. in this evaluation.

## RESULTS AND DISCUSSION

*Fusarium* spp. isolated from damped-off and young root-diseased seedlings are summarized in Table 1. All sampled seedlings with typical damping-off symptoms (hypocotyl decayed and germinant bent over at groundline) were colonized with *Fusarium* spp. Most were either colonized with *F. avenaceum* (Fr.) Sacc. or *F. acuminatum* Ell. & Ev.; several from seedlot 4787 were colonized with *F. equiseti* (Corda) Sacc. *F. avenaceum* was also restricted to only one seedlot (6315), whereas *F. acuminatum* was isolated from germinants of all three seedlots. The most commonly isolated species from two month old diseased seedlings was *F. equiseti*. *F. avenaceum* and *F. acuminatum* were isolated less frequently on these older seedlings. Three other *Fusarium* species were isolated from the older seedlings, but not from young damped-off germinants: *F. sambucinum* Fuckel, *F. oxysporum* Schlecht., and *F. proliferatum* (Matsushima) Nirenberg.

Fusaria attacking young germinants were likely seed contaminants (James 1986, 1987). Workers at the nursery routinely immerse containers in hot water, thereby usually eliminating an important source of *Fusarium* inoculum. Both *F. avenaceum* and *F. acuminatum* commonly attacked young germinants, and therefore, were probably carried on Douglas-fir seed. Previous evaluations (James 1987; James and others 1987) have confirmed that these species may commonly be seedborne. *F. equiseti* may also attack young germinants, especially on some seedlots and is likely also seedborne. This species also persists on seedlings or is periodically introduced later during the growing season. The diversity of *Fusarium* spp. on seedling roots increases as seedlings become older, probably because of other inoculum sources within or adjacent to greenhouses. Recent evaluations (Dumroese and others 1993; James and others 1991)

have shown that *F. proliferatum* is often the dominant species on roots of diseased and non-diseased container-grown Douglas-fir at the end of the growth cycle. This species seems well adapted to secondary spread during the growing season and is usually well adapted to infect roots of container-grown seedlings. However, this evaluation indicates that *F. proliferatum* is not common on recent germinants and is likely not an important contaminant of Douglas-fir seed. In contrast, this species frequently produced sporodochia on ponderosa pine seed shortly after germination and on young seedlings during their early growth period (James 1992).

Fungal succession on the roots of container-grown Douglas-fir seedlings probably occurs throughout the growth cycle. Initial colonizers may be replaced by others that are better able to compete for available niches and food sources. This succession is probably related to competitive ability of different organisms and relative amounts of available inoculum. Initial colonizers in the genus *Fusarium* are those species carried directly on the seedcoat (James 1986, 1987). As young germinants become established, they may become infected by these seed contaminants. Disease may or may not result, depending on relative susceptibility of infected germinants and level of virulence of associated fusaria. As the seedlings age, their root mycoflora may change. In some cases, pathogenic isolates may be involved, in others, root-colonizing isolates may be competitive saprophytes. Previous work (James and others 1987) has indicated that many roots of container-grown Douglas-fir are infected with various *Fusarium* spp. throughout the growth cycle. When seedlings leave the nursery, their roots are usually infected at some level with these fungi (Dumroese and others 1993; James and others 1987). However, experience indicates that this may not result in decreased seedling performance once outplanted (Dumroese and others 1993).

Table 1--*Fusarium* species associated with post-emergence damping-off and root disease of young Douglas-fir container-grown seedlings at the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Fusarium Species <sup>1</sup>	Percent Seedlings Infected <sup>2</sup>					
	Post emergence --- damping-off ---			----- Root Disease -----		
	Seedlot			Seedlot		
	6315	4787	4876	6315	4787	4876
FAVE	57	0	0	0	33(3)	0
FACU	43	71	100	0	0	67(13)
FEQU	0	29	0	33(7)	100(17)	0
FSAM	0	0	0	67(10)	0	0
FOXY	0	0	0	33(3)	0	0
FPRO	0	0	0	0	33(3)	0
All <i>Fusarium</i>	100	100	100	100(20)	100(23)	67(13)

<sup>1</sup> FAVE = *F. avenaceum*; FACU = *F. acuminatum*; FEQU = *F. equiseti*;  
FSAM = *F. sambucinum*; FOXY = *F. oxysporum*; FPRO = *F. proliferatum*

<sup>2</sup> Percent of sampled seedlings colonized with appropriate fungus. Values in parentheses are colonization intensities of root-diseased seedlings based on percent of root pieces (10 sampled per seedling) colonized.

It would be beneficial to ensure that those fusaria on the roots of seedlings were mostly non-pathogens. There may be opportunities for introducing known saprophytic fusaria or other organisms early in the growth cycle to colonize roots and occupy ecological niches that would otherwise be occupied by pathogens (Elmer and Stephens 1989; Ogawa and Komada 1984; Papavizas 1985). Since it is highly unlikely that *Fusarium* spp. can be excluded from container seedling roots to which they are so well adapted, further work is needed to evaluate opportunities to exclude pathogens by manipulating the mycoflora to the advantage of the seedlings.

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