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THE ROLE OF *FUSARIUM* SPECIES IN THE ETIOLOGY OF CONIFER SEEDLING DISEASES IN FOREST NURSERIES

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ABSTRACT

Fusarium species have long been associated with a multiplicity of diseases of conifer seedlings during various growth phases in forest nurseries. These fungi elicit diseases on both bare root and container-grown seedlings; damage varies widely and often seems related to certain environmental and cultural conditions under which seedlings are grown. The most pathogenic species on bare root seedlings in western North America is usually *F. oxysporum*, although many other species are routinely isolated from both diseased and healthy-appearing seedlings. On container-grown seedlings, *F. proliferatum* is often the most common species causing root diseases. Carefully-controlled pathogenicity tests conducted in the laboratory and within greenhouses indicated that virulence of *F. oxysporum* varies widely and is probably related to genetic differences among strains. Highly-virulent and non-pathogenic isolates were routinely encountered within forest nursery populations. There was much less variability in the virulence of tested *F. proliferatum* isolates. Tests indicated that morphologically-similar *Fusarium* isolates usually have differing potential for causing diseases of conifer seedlings. Tests to locate molecular markers that may be related to differing capacities for eliciting disease are underway.

INTRODUCTION

There have been many reports of diseases of conifer seedlings grown in nurseries that described damping-off and root diseases, often associated with fungi in the genus *Fusarium*. *Fusarium* spp. were often isolated from diseased seedlings and shown to be capable of eliciting disease in controlled pathogenicity tests (Gifford 1911; Hartley and Merrill 1914; Hartley and Pierce 1917). Many early studies were designed to elucidate which nursery environmental factors were conducive or suppressive to disease development (Rathbun 1922; Tint 1945a, 1945b).

However, definitive epidemiological studies of *Fusarium*-associated diseases were not undertaken until the early 1970s by Bloomberg. Several reports (Bloomberg 1971, 1973, 1976) detailed the biology of root disease caused by *F. oxysporum* Schlect. on bare root Douglas-fir (*Pseudotsuga menziesii* Franco) seedlings in British Columbia,

Canada. This work provided the basis for understanding the epidemiology of *F. oxysporum*; a model outlining infection processes and disease development was formulated (Bloomberg 1979). This model has helped nursery growers develop more effective strategies for combating *Fusarium*-associated disease in bare root nurseries.

Fusarium spp. cause several different diseases on conifer seedlings. These fungi decay seed, thus preventing germination (Bloomberg 1981, Huang and Kuhlman 1990; Matuo and Chiba 1966). The also induce damping-off either before (pre) or after (post) seedling emergence (Bloomberg 1981; Hartley et al. 1914; Lock 1973). Damped-off seedlings are usually attacked at the radicle or main stem when tissues are succulent (Spaulding 1914). Once seedling stems lignify a few weeks after emergence, they are no longer susceptible to damping-off (Rathbun-Gravatt 1925; Spaulding 1914). *Fusarium* spp. also cause root disease, primarily during the first growing season on bare root seedlings (James 2001) and throughout the growth cycle on container-grown seedlings (James et al. 1987); *Fusarium*-associated diseases are rare during the second growing season (Enebak et al. 1990; Sinclair et al. 1975). Several *Fusarium* species are also capable of causing stem cankers above the groundline which may expand and eventually girdle seedlings (Brownell and Schneider 1985; Cooley 1983; Hansen and Hamm 1988; James 2003b). There have been a few reports of *Fusarium* spp. causing top blight, resulting in apical dieback of seedlings (Bloomberg 1981; Hartley et al. 1914; James 2003b; Matuo and Chiba 1966). In some cases, etiology of canker and top blight diseases may be associated with other microorganisms in addition to *Fusarium* spp.

Most conifer species are susceptible to infection by *Fusarium* spp. (Bloomberg 1981; James 1985; James and Gilligan 1984). However, in western North America, most damage occurs on Douglas-fir, western larch (*Larix occidentalis* Nutt.), true firs (*Abies* spp.), ponderosa pine (*Pinus ponderosa* Laws.) sugar pine (*Pinus lamertiana* Dougl.) and Engelmann spruce (*Picea engelmanni* Parry)(Bloomberg 1981; Cooley 1983; Hansen and Hamm 1988; James 1985; James and Gilligan 1985, 1988a; James et al. 1987). Other conifer species usually less affected include lodgepole pine (*Pinus contorta* Dougl.) and western white pine (*Pinus monticola* Dougl.)(James 1990a, 1990c, 1991a, 1991b).

Several *Fusarium* spp. have routinely been isolated from diseased conifer seedlings. *Fusarium oxysporum* is often the major pathogen of bare root seedlings in western North America (Bloomberg 1971, 1973, 1976, 1979; Brownell and Schneider 1985; Cooley 1983; James and Gilligan 1988b; James et al. 2000; Tint 1945a). This species may also be common on container-grown seedlings, particularly early in the growth cycle as a result of contaminated seed (James 1986, 1987; James et al. 1986, 1989a, 2000; Mousseaux et al. 1998). Other *Fusarium* species commonly isolated include *F. proliferatum* (Matsushima) Nirenberg, especially on container-grown seedlings (James 1985; James and Gilligan 1985; James et al. 1987, 1995, 1997), *F. acuminatum* Ell. & Ev., *F. solani* (Mart.) Appel & Wollenw. and *F. sporo-*

trichioides Sherb.(James 2000; James and Gilligan 1984, 1988a; James and Perez 1999, 2000). Other *Fusarium* species less commonly associated with diseased conifer seedlings include *F. avenaceum* (Fr.) Sacc., *F. sambucinum* Fuckel, *F. equiseti* (Corda) Sacc., *F. culmorum* (W.G. Smth) Sacc., *F. poae* (Peck) Wollenw.) and *F. tricinctum* (Corda) Sacc. (James 1990b, 1990d, 1992, 1993). It is likely that not all of these species are pathogenic on conifer seedlings, even though they may frequently colonize seedling tissues.

A major problem in dealing with *Fusarium* diseases is conifer seedling infection without disease symptoms being expressed (Bloomberg 1971, 1973; Hartley et al. 1914; James et al. 1987). Surveys indicated that many bare root (James and Gilligan 1988b) and container-grown (James and Gilligan 1985, 1988a; James et al. 1987) seedlings may be infected with *Fusarium* spp. even though disease symptoms are lacking. Because of this problem, tests were conducted over several years to elucidate the potential of the more commonly-isolated species of *Fusarium* to cause disease under both greenhouse and controlled laboratory conditions (James 2000; James and Gilligan 1984; James and Perez 1999, 2000; James et al. 1986 1989a, 1997, 2000). From this work, a clearer understanding of the role of *Fusarium* species in the etiology of conifer seedling diseases was obtained.

MATERIALS AND METHODS

Tests were conducted in greenhouses as well as under more controlled conditions in the laboratory. All tests were conducted on Douglas-fir germinants or older seedlings. For all tests, fungal inoculum was prepared using the technique of Miles and Wilcoxin (1984). This technique uses perlite, an inert, inorganic, siliceous rock of volcanic origin, commonly used in potting mixtures, as the matrix for fungal growth. To provide nutrients for fungal growth, cornmeal (150g) was moistened with warm 1% potato dextrose agar (PDA)(300ml) and added to perlite (75g). Perlite/cornmeal/PDA mixtures were inoculated with 10ml spore suspensions of the *Fusarium* isolates to be tested (prepared by adding sterile, distilled water to 14-day-old PDA cultures) and incubated in the dark at about 24°C for at least 21 days, after which the fungus had thoroughly colonized the perlite/cornmeal substrate. After incubation, inoculum was dried in open petri plates within a cabinet. After 5-7 days, inoculum was dry and uncontaminated because the substrate was completed colonized by the test fungus. Inoculum was stored in plastic vials under refrigeration until needed, remaining viable for at least 2 years. In all tests, when inoculum became wet, test fungi became active.

Greenhouse Tests:

Greenhouse pathogenicity tests were conducted on new germinants and seedlings either 2- or 5-months old. Two isolates each of two *Fusarium* species (*F. oxysporum* and *F proliferatum*) were tested for their ability to infect, colonize, and induce disease on young seedlings. Tested isolates were from both diseased and healthy-appearing conifer seedlings or from diseased seeds from north Idaho nurseries.

Germinant tests: Metal trays (38cm x 23cm x 5cm) were filled with a 1:1 peat/vermiculite commercial growing medium, autoclaved for 60 min. at 121°C, and allowed to cool. Inoculum was mixed thoroughly into the autoclaved medium at either a 1:25 or 1:50 (w/w) concentration. Controls consisted of autoclaved growing medium amended with uninoculated perlite at the same two treatment concentrations. The inoculum-growing medium mixture was moistened with sterile distilled water until the mixture was at field capacity.

Douglas-fir seeds were treated using standard bleach (sodium hypochlorite) (Wenny and Dumroese 1987), then immersed for 3 min. in a 70% v/v solution of ethanol. Treated seeds were placed in a running water rinse for 48 hrs. After 28 days of cold stratification at 2°C, 200 seeds were sown within each tray containing the inoculum-growing medium mixture and covered with about 0.5cm of grit.

Germination and seedling emergence was monitored in each tray. Post-emergence damping-off was determined by examining trays for fallen seedlings and seedlings with stem lesions. Damped-off seedlings were carefully removed and replaced with toothpicks. The seedlings were washed carefully, surface sterilized in a 10% bleach solution (0.525% aqueous sodium hypochlorite) and incubated on a selective 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 *Fusarium* isolates were compared with inoculated isolates to confirm they were the same. Experiments were terminated when germination was considered complete (27 days after sowing). Upon completion of experiments, 10 non-diseased seedlings (if available) were collected from each tray for root isolations using the procedures described above. Emerging fusaria were compared with inoculated isolates to determine if inoculated isolates had invaded the non-diseased seedlings.

Young (2 month-old) seedling tests: Seedlings to be tested were initially grown in Spencer-LaMeire Root Trainers® for 7 weeks prior to inoculation. Seedlings were carefully removed from their containers to minimize root damage. Seedling roots were washed thoroughly to remove adhering particles of growing media, and the seedlings were carefully repotted into Ray Leach® pine cells (66 cm^3) with an inoculum-growing medium mixture (1:50 w/w). Twenty-five seedlings were tested per fungal isolate. Controls consisted of repotted seedlings in an uninoculated perlite-soil mixture. Seedlings were watered with fine-mist, overhead irrigation within the greenhouse as needed.

Severity of root disease symptoms was rated using a five-point numerical system based on the extent of foliar chlorosis and necrosis. When seedlings were considered dead, they were carefully removed from containers. Their roots were thoroughly washed to remove growing medium and aseptically dissected into pieces about 2-3 mm in length. Root pieces were incubated on Komada's medium as described above. Emerging fusaria were compared with the inoculated isolates to confirm they were the same.

Experiments were terminated 84 days after inoculation. At that time, all remaining seedlings were rated for severity of disease symptoms, removed from their containers, and their roots analyzed for presence of *Fusarium* as described above. Comparisons were made between emerging fusaria and inoculated isolates to confirm infection by tested isolates.

Older (5 month-old) seedling tests: Seedlings were grown for 5 months in Ray Leach® pine cells; test seedlings were carefully removed from their containers and their roots thoroughly washed to remove as much growing media as possible. They were repotted into Ray Leach® super cells (144 cm3) with the inoculum-growing medium mixture (1:50 w/w). Ten seedlings per isolate were tested. Controls consisted of repotted seedlings in uninoculated perlite-soil mixture.

Seedlings were watered and monitored for foliar indications of root disease. Experiments were terminated after 68 days. Dead seedlings were evaluated for presence of inoculated isolates as described above. At the end of each experiment, all surviving seedlings were rated for extent of disease symptoms using the same rating system for younger seedlings and carefully removed from their containers. For each seedling, 10 lateral roots were randomly selected and pieces 2-3 mm in length were aseptically severed from selected roots. Root pieces were incubated on Komada's medium as described above. Emerging fusaria were compared with inoculated isolates to confirm root infection by test isolates.

Laboratory Tests:

Laboratory pathogenicity tests were conducted using a technique for rapid assessment of virulence (James 1996). The basic approach was to expose Douglas-fir germinants to fungal isolates and record production of disease symptoms. *Fusarium* isolates from 5 species (*F. oxysporum, F. proliferatum, F. solani, F. sporotrichioides*, and *F. acuminatum*) were tested in the laboratory.

Twenty-four vials (25ml capacity) were used to test each fungal isolate. Each vial was filled to about 2/3 capacity with dried 1:1 (v/v) coir pith medium (Grace/Sierra Horticultural Products, Milpitas, CA). This medium has periodically been used by some nursery growers to produce many different container-grown plants including conifer seedlings (Martinez 1995). Vials with media were autoclaved at 121°C for 60 min. and cooled before being used in tests.

Perlite/cornmeal/PDA inoculum was ground to a fine powder with mortar and pestle and 0.05g of the powder was added to each vial containing dried coir media. This resulted in an approximately 1:50 (w/w) mixture of inoculum to media. Inoculum was distributed throughout the media by shaking. Seeds were treated as described above, stratified, and incubated on moistened filter paper within petri dishes to induce germination. One recently-germinated seed (germinant) was carefully placed into each vial with its primary root oriented downward into the medium. Sterile water (4ml) was added to each vial with caps replaced loosely to allow aeration. Controls consisted of 24 vials with non-inoculated perlite added instead of inoculum.

Vials containing germinants were incubated at about 20-24°C on a lab bench with 8-12 hr. daily fluorescent light. Each isolate was evaluated for its ability to cause germinant disease within 14 days. After 3 days, germinants were first checked for disease and evaluated daily thereafter. Standard post-emergence damping-off was the most common disease encountered (James 1996). A wet-type decay sometimes occurred where the root was attacked but the above-ground portion of the germinant was not affected (James 1996; James and Perez 1999, 2000). Diseased seedlings were removed when discovered, their roots thoroughly washed, and incubated on Komada's medium as describe above for re-isolation of inoculated isolates. After 14 days, all surviving germinants were examined to determine if their roots grew to the bottom of the vial. Roots of surviving seedlings were also analyzed for infection by inoculated isolates.

A numerical rating system for isolate comparisons was used which awards points based on duration of germinant survival, occurrence and type of disease, re-isolation of inoculated test isolates, and primary root growth within vials (James 1996). The range of possible points was 3-23, with higher point values reflecting less virulence. To convert points to a score (virulence rating) in which higher numbers represented greater virulence, a reciprocal rating system was used (James 1996). In this system, the maximum score was 100 and the minimum score was zero.

Average virulence ratings of isolates were used to compare isolates. Based on previous experience (James 2000; James and Perez 1999, 2000; James et al. 1997, 2000), highly virulent isolates exhibited scores of 80-100, moderately virulent isolates from 60-80, isolates with low virulence from 40-60, and isolates with average scores below 40 were considered non-pathogenic. In tests involving *F. oxysporum, F. solani and F. acuminatum* isolates, average virulence scores were compared using one-way analysis of variance; comparisons were made between nurseries (*F. oxysporum*) and among isolate sources (soil, seedling roots, etc.). Significant means (P=0.05) were separated using Tukey's HSD test.

RESULTS

Greenhouse Tests: Examples of greenhouse test results are summarized in tables 1-3 for two isolates each of *F. proliferatum* and *F. oxysporum*.

Germinant Test: Results from tests involving young Douglas-fir germinants (table 1) indicated that the two tested *F. proliferatum* isolates severely reduced seed germination (in comparison with controls) and seedling establishment as well as caused very high levels of post-emergence damping-off. The two *F. oxysporum* isolates also greatly reduced seed germination and caused post-emergence damping-off, but at levels much less than those caused by *F. proliferatum*. Differences in effects on seed germination and damping-off between the two inoculum levels (1:25 vs. 1:50) were not apparent.

Young (2 month-old) seedlings: Inoculation results on young seedlings (table 2) indicated that the two *F. proliferatum* isolates did not vary greatly in their ability to colonize and cause

extensive disease on seedlings. They were much more virulent that the two *F. oxysporum* isolates tested, causing higher seedling mortality and greater average disease severity ratings. Both species readily colonized all seedling roots, but the differences were manifested in level of disease production.

Older (5 month-old) seedlings: Average disease ratings were higher for the two tested *F. proliferatum* isolates than those for the two *F. oxysporum* isolates on older seedlings in greenhouse inoculation tests (table 3). Percent root colonization was likewise higher on seedlings inoculated with *F. proliferatum*. There were low levels of disease on some control seedlings, mostly due to infection by *F. proliferatum*, which apparently spread from inoculated test seedlings to nearby control seedlings.

Table 1. Effects of selected *Fusarium proliferatum* and *F. oxysporum* isolates of germination of Douglas-fir seed and post-emergence damping-off of young germinants in the greenhouse.

Species	Isolate	Inoculum Concentration	Percent Germination	Percent Damping-off
Fusarium proliferatum	No. 1	1:25	15	97
		1:50	15	97
Fusarium proliferatum	No. 2	1:25	6	100
		1:50	17	94
Fusarium oxysporum	No.1	1:25	25	33
		1:50	43	32
Fusarium oxysporum	No.2	1:25	29	35
		1:50	24	46
Controls		1:25	72	1
		1:50	69	1

Table 2. Effects of two *Fusarium proliferatum* and *F. oxysporum* isolates on disease severity in 2 month-old inoculated Douglas-fir seedlings in the greenhouse.

Species	Isolate	Percent Mortality	Average[1] Disease Rating	Percent Root Colonization[2]
Fusarium proliferatum	No. 1	92	4.7	100
	No. 2	96	4.9	100
Fusarium oxysporum	No. 1	24	2.8	100
	No. 2	32	3.0	100
Controls		0	1.6	0

[1] Rating system from 1-5; higher numbers indicate more severe disease symptoms.
[2] Seedlings not killed by *Fusarium* within 84 days of inoculation; root colonization is the percent of sampled root system colonized by inoculated isolates.

Table 3. Effects of two *Fusarium proliferatum* and *F. oxysporum* isolates on disease severity in 5 month-old Douglas-fir seedlings in the greenhouse.

Species	Isolate	Average Disease Rating[1]	Percent Root Colonization[2]
Fusarium proliferatum	No. 1	3.0	85
	No. 2	3.5	99
Fusarium oxysporum	No. 1	2.0	77
	No. 2	1.6	87
Controls		2.0	25

[1]Rating system from 1-5; higher numbers mean more severe disease symptoms.
[2]Seedlings not killed by *Fusarium* within 68 days of inoculation; root colonization is percent of sampled root system colonized by inoculated isolates [for controls, percent of sampled root system colonized by any *Fusarium* spp.].

Laboratory Tests:

Results from screening 179 *F. oxysporum* isolates for virulence in the laboratory are summarized in tables 4-6. There were generally little differences in level of virulence between isolates obtained from nursery soil and those isolated from conifer seedling roots (table 4), although soil isolates were significantly (P=0.05) more virulent than root isolates at one nursery. *Fusarium oxysporum* isolates from roots of diseased seedlings were consistently more virulent than those obtained from the roots of healthy seedlings at both sampled nurseries (table 5). There was generally a wide range of virulence of the *F. oxysporum* isolates obtained from different sources and nurseries (table 6). About 17% of the tested isolates would be considered highly virulent and nearly the same percent were considered non-pathogenic. Nearly half of the tested isolates were either highly or moderately virulent in carefully-controlled laboratory pathogenicity tests.

In contrast, the vast majority of the 43 *F. proliferatum* isolates evaluated in similar tests were found to be highly virulent; nearly 98% of the tested isolates were either highly or moderately virulent (table 7). No *F. proliferatum* isolate tested was non-pathogenic on young Douglas-fir germinants, regardless of its isolation source.

Of the 47 *F. solani* isolates evaluated, most obtained from nursery soil were non-pathogenic, whereas most from roots of either healthy or diseased seedlings exhibited low virulence (table 8). Although a very few of the isolates from seedling roots were either moderately or highly virulent, the majority exhibited either low virulence or were non-pathogenic.

Isolates of both *F. acuminatum* and *F. sporotrichioides* evaluated for virulence on young Douglas-fir germinants were mostly non-pathogenic (tables 9 and 10). A few *F. acuminatum* isolates exhibited either low or moderate virulence and only one *F. sporotrichioides* isolate had a low level of virulence.

Table 4. Comparisons of average virulence scores for *Fusarium oxysporum* isolates obtained from nursery soil and conifer seedling roots at two nurseries in the inland Pacific Northwest.

Isolate Source	Number Tested	Nursery No. 1	Nursery No. 2	Both Nurseries
Soil	129	57.6A[1]	63.6A[1]	59.5A[1]
Root Tissue[2]	50	58.4A	51.8B	54.8A

[1] Within each column, means followed by the same capital letter are not significantly different (P=0.05) using Tukey's HSD.
[2] Includes healthy and diseased seedlings.

Table 5. Comparisons of average virulence scores of *Fusarium oxysporum* isolates obtained from roots of healthy and diseased conifer seedlings at two nurseries in the inland Pacific Northwest.

Isolate Source	Number Tested	Nursery No. 1	Nursery No. 2	Both Nurseries
Healthy Roots	33	45.6B[1]	51.7B[1]	50.2B[1]
Diseased Roots	17	66.5A	56.2A	64.1A

[1] Within each column, means followed by the same capital letter are not significantly different (P=0.05) using Tukey's HSD.

Table 6. Virulence of tested *Fusarium oxysporum* isolates from inland Pacific Northwest nurseries.

Isolate Source	Percent of Isolates within Virulence Category				
	No. Tested	High	Medium	Low	Non-Pathogenic
Soil	129	19.4	28.7	34.9	17.0
HealthyRoots	33	6.1	27.3	36.4	30.2
Dis. Roots	17	23.5	23.5	53.0	0
All Isolates	179	17.3	27.9	36.9	17.9

Table 7. Virulence of tested *Fusarium proliferatum* isolates from inland Pacific Northwest nurseries.

Isolate Source	Percent of Isolates within Virulence Category				
	No. Tested	High	Medium	Low	Non-Pathogenic
Containers[1]	16	100	0	0	0
HealthyRoots	5	100	0	0	0
Dis. Roots	19	63.2	31.5	5.3	0
Insects[2]	3	100	0	0	0
All Isolates	43	83.7	14.0	2.3	0

[1] Styroblock containers from several greenhouse nurseries.
[2] Collected directly from adult fungus gnats (*Bradysia* spp.- Diptera: Sciaridae).

Table 8. Comparisons of average virulence scores of *Fusarium solani* isolates obtained from nursery soil and roots of healthy and diseased conifer seedlings in the inland Pacific Northwest.

Isolate Source	Number of Isolates Tested	Virulence Scores	
		Average[1]	Range
Soil	35	36.2B	20.0-76.1
Healthy Roots	6	46.3A	26.0-85.0
Diseased Roots	6	47.4A	25.5-97.1
All Isolates	47	39.0	20.0-97.1

[1]Within this column, means followed by the same capital letter are not significantly different (P=0.05) using Tukey's HSD.

Table 9. Comparison of average virulence scores of *Fusarium acuminatum* isolates obtained from nursery soil, containers, insects, seeds, and roots of healthy and diseased conifer seedlings in the inland Pacific Northwest.

Isolate Source	Number of Isolates Tested	Virulence Scores	
		Average[1]	Range
Soil	6	29.9A	23.9-34.0
Healthy Roots	11	33.8A	17.3-70.8
Diseased Roots	12	34.8A	17.7-58.5
Seeds	2	32.5A	26.5-38.5
Insects[2]	1	31.3A	31.3
Containers[3]	1	32.7A	32.7
All Isolates	33	33.2	17.3-70.8

[1]Within this column, means followed by the same capital letter are not significantly different (P=0.05) using Tukey's HSD.
[2] Collected directly from adult fungus gnats (*Bradysia* spp.- Diptera: Sciaridae).
[3] Styroblock containers from several greenhouse nurseries.

Table 10. Virulence of tested *Fusarium sporotrichioides* isolates from inland Pacific Northwest nurseries.

Isolate Source	Percent of Isolates within Virulence Category				
	No. Tested	High	Medium	Low	Non-Pathogenic
Soil	1	0	0	0	100
Healthy Roots	3	0	0	0	100
Diseased Roots	7	0	0	0	100
Seeds	1	0	0	0	100
Containers[1]	4	0	0	25	75
Growing Media[2]	4	0	0	0	100
All Isolates	21	0	0	4.8	95.2

[1]Styroblock containers from several greenhouse nurseries.
[2]Standard peat/vermiculite growing media used to produce container seedlings.

DISCUSSION

Greenhouse and laboratory pathogenicity tests revealed a wide range of disease-causing ability within populations of *F. oxysporum* from forest nurseries in the inland Pacific Northwest. Higher disease levels were evident on younger seedlings; as seedlings became older, they became less susceptible to disease, although these older seedlings were often infected by most tested isolates. *Fusarium oxysporum* caused both pre- and post-emergence damping-off as well as root disease of older seedlings in these tests, similar to the types of disease common in both bare root and container nurseries (Bloomberg 1971, 1981; James et al. 1987, 1991, 2000). Although isolates of *F. oxysporum* often appear morphologically similar (James et al. 1989, 1991; Nelson et al. 1983), they display a wide range of virulence potential and differences in virulence do not appear related to morphological characters (Awuah and Lorbeer 1988; Gordon and Martyn 1997; Gordon and Okamoto 1992; Tint 1945a, 1945b). Previous work on isolates of this species from agricultural crops indicated that there may be molecular differences among isolates and these differences may be related to pathogenicity (Appel and Gordon 1995; Bentley et al. 1998; Gordon and Okamoto 1992; Kelly et al. 1994). There are definite portions of the *F. oxysporum* population that always behave as non-pathogens (Correll et al. 1986; Gordon and Martyn 1977; Kistler 1997), and some of these isolates have been useful as potential biological control agents to prevent disease by pathogenic isolates (Alabouvette et al. 1993; Amir and Alabouvette 1993; Blok et al. 1997; Damicone and Manning 1982). Work is currently in progress to evaluate the genetic diversity of *F. oxysporum* populations from one forest nursery in northern Idaho using AFLP analyses. From this work, it is hoped that specific molecular markers may become available to characterize highly virulent isolates within the population. In this way, it will be easier to predict disease potential from soil and seedling assays for this very important, potentially-pathogenic fungal species.

Fusarium proliferatum has routinely been isolated from root diseased container-grown seedlings at many nurseries for several years (James 1985; James and Gilligan 1985; James et al. 1987, 1995, 1997). This species is less variable than *F. oxysporum* morphologically, and is characterized by the profuse production of microconidial chains (James et al. 1989b; Nelson et al. 1983). This provides the means for rapid buildup and spread of the fungus, particularly within greenhouses (Elmer 1990; James et al. 1995, 1997). As a result, roots of many container-grown seedlings become infected by the end of the growth cycle, even though they may not display disease symptoms (Dumroese et al. 1993; James 1997; James and Gilligan 1988a; James et al. 1995). Greenhouse and laboratory pathogenicity tests indicated that nearly all of the tested isolates were highly virulent on Douglas-fir germinants and young seedlings. However, disease severity on older seedlings appeared less dramatic; apparently seedlings are capable of developing resistance to disease caused by this fungal species as they age (James 1997). Fortunately, *F. proliferatum* is not an abundant contaminant of conifer

seed (James 1986, 1987, 1990b), nor is it very common in forest nursery soil (James 2002a, 2002b, 2003a). Many container-grown seedlings become infected with *F. proliferatum* during the growth cycle (James 1997). When infected seedlings are outplanted onto typical forest sites, *Fusarium* tends to decline on root systems, often being replaced by other mycoflora, particularly ectomycorrhizal symbionts (Dumroese et al. 1997; Sinclair et al. 1975; Smith 1967). Therefore, high levels of nursery infection by *F. proliferatum* do not necessarily result in severe disease, nor does it usually adversely affect out-planting performance (Dumroese et al. 1997).

The other three *Fusarium* species evaluated for virulence potential are routinely isolated from forest nursery soil and the roots of both healthy and diseased conifer seedlings (James 2002a, 2002b, 2003a; James and Gilligan 1988a, 1988b). *Fusarium solani* is particularly well adapted to survival in soil and is commonly associated with *F. oxysporum* on roots of bare root seedlings (James 2002a, 2002b, 2003a). *Fusarium acuminatum* may be very common in soil and on the roots of bare root seedlings, but is also routinely isolated from container-grown seedlings (James 1992, 1993). *Fusarium sporotrichioides* is encountered less frequently, but may be associated with important conifer seedling diseases in forest nurseries (James 2003b; Rathbun-Gravatt 1925, 1931). Most tested isolates of these three species exhibited either low virulence or were considered non-pathogenic on young germinants. Therefore, they may not be important pathogens, at least during the seed germination/damping-off stage of seedling development. However, it is possible that there are some isolates, not included in these tests, that are capable of causing severe disease, especially under highly conducive environmental conditions (James et al. 1991).

There are other *Fusarium* species that need to be evaluated for their disease-causing potential within forest nurseries. A combination of both greenhouse and laboratory tests are necessary to better clarify the role of *Fusarium* species in causing important nursery diseases. While the majority of isolates encountered from standard soil assays and root isolations may have low disease potential, there is undoubtedly some portion of the population that can cause important diseases. These highly virulent pathogens need to be identified, particularly using recent molecular genetic technology, to help growers predict disease potential and, as a result, institute effective integrated pest management actions to reduce disease impacts in their nurseries.

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