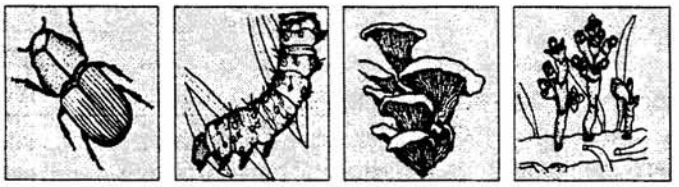
Forest Health Protection



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PATHOGENIC CHARACTERISTICS OF *FUSARIUM SPOROTRICHIOIDES* ISOLATED FROM INLAND PACIFIC NORTHWEST FOREST NURSERIES

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ABSTRACT

Twenty-one isolates of *Fusarium sporotrichioides* obtained during investigations of conifer seedling diseases in northern Rocky Mountain forest nurseries were evaluated for pathogenicity on young Douglas-fir germinants in controlled laboratory tests. All but one of the isolates were non-pathogenic on germinants; the other isolate displayed low levels of virulence. Two isolates of *F. proliferatum* had moderate and low virulence in pathogenicity tests run concurrently with the *F. sporotrichioides* test. One potential biological control isolate of *F. oxysporum* was also non-pathogenic on germinants. Implications of these results on management of conifer seedling diseases are discussed.

INTRODUCTION

Fusarium spp. are commonly associated with important diseases of container and bareroot conifer seedlings in forest nurseries throughout North America (James and others 1991). Diseases can be especially severe within greenhouses because seedling growing regimes are conducive to infection and buildup of *Fusarium* spp. (James 1984). Sometimes, fusaria cause devastating losses in container nurseries because pathogens spread quickly, cause disease symptoms rapidly, and growers fail to respond quickly to the disease (James and Gilligan 1984).

Investigations of *Fusarium*-associated diseases of forest seedlings often resulted in several different *Fusarium* species associated with diseased symptoms (James and others 1989a). Some species, such as *F. oxysporum* Schlecht. and *F. proliferatum* (Matsushima) Nirenberg are known to be consistently associated with various diseases and capable of eliciting disease symptoms during carefully controlled pathogenicity tests (James and others 1988, 1989b, 1995). However, other *Fusarium* species are isolated less frequently. One of these is *F. sporotrichioides* Sherb., a species isolated from both diseased and non-diseased seedlings (Hartley and others 1919; James 1985; Rathbun 1922), conifer seed (James 1987; Mittal and Wang 1987, 1989, 1993; Singh and Mittal 1989), container growing media (James 1992), containers (James and others 1988b), and nursery soil (James and Beall 1999). Some researchers report that *F. sporotrichioides* is a very aggressive pathogen of conifer seedlings (Hartley and others 1918; Mittal and Wang 1993; Pomerlau 1934; Rathbun 1922; Rathbun-Gravatt 1925, 1931; Seemuller 1968). Although *F. sporotrichioides* is often associated with other *Fusarium* spp. on diseased conifer

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seedlings in northern Rocky Mountain nurseries (James and others 1989a), its importance as a pathogen required investigation. Therefore, to help elucidate its role as a potential pathogen of conifer seedlings, we conducted pathogenicity tests on young Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco) germinants with selected *F. sporotrichioides* isolates.

MATERIALS AND METHODS

During routine investigations of conifer seedling diseases at inland Pacific Northwest nurseries over a 9 year period, 21 isolates of *F. sporotrichioides* were collected. Isolates were obtained from conifer seed, healthy and diseased seedlings, container growing media, containers, and nursery soil (see appendix for detailed descriptions). Samples were incubated on a selective agar medium for *Fusarium* (Komada 1975) and isolates single spored and transferred to carnation leaf (Fisher and others 1982) and potato dextrose agar (PDA) for identification. Isolates were classified as *F. sporotrichioides* if three distinct morphological types of microconidia (oval, pear-shaped, spindle-shaped) on either mono- or poly-phialides were present (Nelson and others 1983; Van Wyk and others 1990). Cultures on PDA typically produced extensive white aerial mycelium with a carmine red under surface. Abundant chlamydospores grew either singly, in chains, or in clumps. Axenic cultures, maintained on carnation leaves in sterile, distilled water, remained viable for several years.

We conducted pathogenicity tests using a technique for rapid laboratory assessment of virulence (James 1996; see appendix). Tests were conducted on the 21 *F. sporotrichioides* isolates plus two isolates of *F. proliferatum*, a species usually quite virulent in laboratory tests (James and others 1995, 1997), and one *F. oxysporum* isolate considered non-pathogenic and capable of biological control of pathogenic *F. oxysporum* strains (Lemanceau and others 1992, 1993a).

The average virulence rating for germinants tested against a particular isolate was used to compare isolates. Based on previous experience (James and others 1995, 1997), highly virulent isolates exhibit average scores of 80-100; moderately virulent isolates: 60-80; low virulent isolates: 40-60; isolates with average scores below 40 are considered non-pathogenic.

Average virulence scores for all isolates and controls were compared using a one-way analysis of variance. Significant mean differences (P=0.05) were located with Duncan's Multiple Range Comparison Test.

RESULTS AND DISCUSSION

All but one of the *F. sporotrichioides* isolates tested were considered non-pathogenic on Douglas-fir germinants (figure 1; see appendix for individual isolate scores). The exception (isolate 9307D) had a significantly higher average virulence rating (P=0.05) than the other isolates, but was still considered weakly virulent on germinants. For comparison, the 2 tested *F. proliferatum* isolates displayed either low or moderate virulence, although their average scores were higher than any of the *F. sporotrichioides* isolates tested. The *F. oxysporum* isolate was non-pathogenic as well, which was expected because this isolate is non-pathogenic on several agricultural plants and may elicit biological control against pathogenic strains of *F. oxysporum* on some crops (Lemanceau and others 1992, 1993). Summaries of Douglas-fir germinant infection, disease caused by tested *Fusarium* isolates, and average length of time germinants remained alive during the test are given in table 3 - appendix. Most germinants were infected by *F. sporotrichioides* test isolates, even though many germinants did not become diseased. Most germinants remained alive throughout the experiment.

We know that Douglas-fir is usually one of the most susceptible conifer species to *Fusarium* diseases (James 1984; James and others 1988b, 1991). Many pathogenic isolates of *Fusarium* do not normally cause disease on specific conifer species, but rather are usually capable of attacking several different species when environmental conditions are conducive (Hartley and others 1918; James and others 1991).

Fusarium sporotrichioides may be an important pathogen of conifer seedlings (Hartley and others 1918; Mittal and Wang 1993; Rathbun 1922; Rathbun-Gravatt 1925, 1931; Tint 1945) and other plants including water milfoil (Andrews and Hecht 1981), various grasses and cereals (Chelkowski and others 1989; Gordon and Sprague 1941; Stack and McMullan 1985; Sturz and Bernier 1991), corn (Neish and others 1983; Vargo and Baumer 1986) and parsnip (Desjardins and others 1989). One of its most important characteristics is its ability to produce powerful mycotoxins called trichothenes which may be extremely toxic to plants and animals (Atanassov and others 1994; Fekete and others 1993; Logreico and others 1990; Moss and Frank 1985; Subramanian 1983). Therefore, we expected isolates of *F. sporotrichioides* to be much more virulent on conifer germinants, especially under conditions of our tests which are conducive to disease (James 1996; James and others 1997). However, we found that most *F. sporotrichioides* isolates were not pathogenic and are probably secondary colonizers of organic matter. Therefore, it is likely that *F. sporotrichioides* is less important a pathogen as other *Fusarium* spp. commonly associated with conifer seedling diseases.

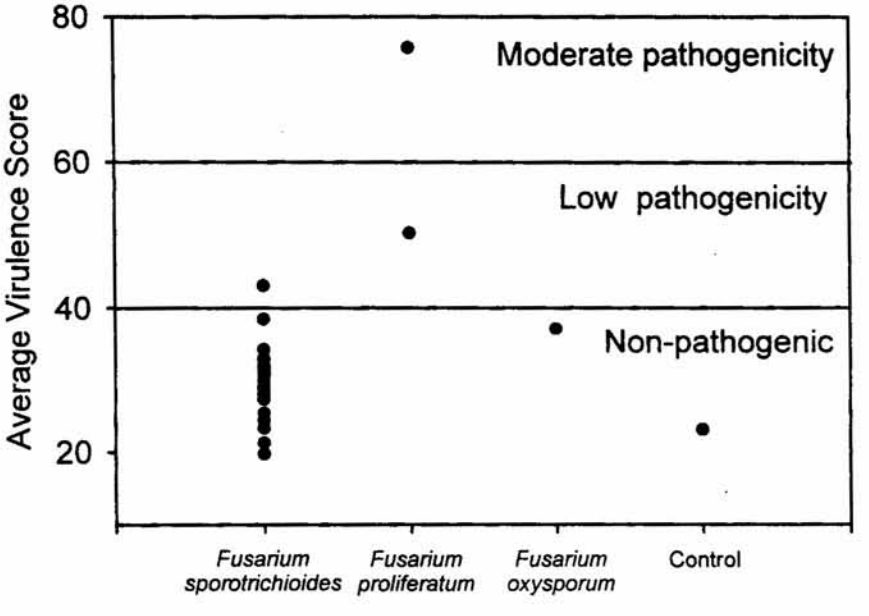

Figure 1. Comparison of average virulence scores of isolates of *Fusarium sporotrichioides, F. proliferatum, F. oxysporum* and the control from forest nurseries on Douglas-fir germinants.

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APPENDIX

Description of laboratory technique to evaluate virulence (see James 1996):

The basic approach of pathogenicity tests was to expose young Douglas-fir germinants to tested fungal isolates and record production of disease symptoms. Cornmeal-perlite inoculum was prepared for each tested isolate using the techniques of Miles and Wilcoxsin (1984). Perlite, an inert, inorganic, siliceous rock of volcanic origin commonly used in potting mixtures, was the matrix for fungal growth. Yellow cornmeal (150g) was moistened with 300ml warm 1 percent potato dextrose agar (PDA), to which 75g of perlite was added. The mixture was placed into 25ml glass vials to about two-thirds capacity which were autoclaved for 60 minutes at 121°C. After cooling, vials were inoculated with about 10ml of a spore suspension of each test fungus. The spore suspension was produced by adding sterile, distilled water to 14-day-old cultures grown on PDA. Vial caps were left loose to allow aeration and incubated in the dark for at least 21 days, after which the fungus had thoroughly colonized the perlite/cornmeal mixture. After incubation, inoculum was removed from vials and dried for 5-7 days in open petri plates within a cabinet. Inoculum did not become contaminated with other organisms during drying because the food base was completely colonized by the test fungus (Miles and Wilcoxin 1984). Once dry, inoculum was stored in plastic vials and refrigerated until needed. This type of inoculum remained vi-

able in previous tests for at least 2 years (James 1996).

Twenty-four vials (25ml capacity) were used to test each fungal isolated. Each vial was filled to about 2/3 capacity with dried coconut-vermiculite media (Grace/Sierra Horticultural Products, Milpitas, CA). This media was made up of 50 percent coir pith (coconut fiber) and 50 percent vermiculite (v/v) and has periodically been used by some growers to produce many different container-grown plants including conifer seedlings. Vials with media were autoclaved at 121°C for 60 minutes and cooled before being used in tests.

We used one Douglas-fir seedlot with high germination capacity and energy for all tests. Seeds were initially soaked in an aqueous solution of sodium hypochlorite (2 parts bleach with 3 parts water) for 10 minutes (Wenny and Dumroese 1987), rinsed 48 hours in running tap water, and stratified 21 days at 2-3°C. After stratification, seeds were placed on filter paper moistened with sterile water, in sterile petri plates. Seeds were incubated under 12-hour diurnal fluorescent light cycles at about 24°C and monitored daily for germination. Seeds were considered germinated when their primary root was at least 3mm long.

Perlite/cornmeal inoculum for each test isolate was ground to a fine powder with mortar and pestle and 0.05g of the powder was added to each vial containing dried media. This resulted in an approximately 1:50 w/w mixture of inoculum to media. Inoculum was distributed throughout the media by shaking. One recently germinated seed (germinant) was carefully placed into each vial with its primary root placed downward into the media. Four ml sterile water was added to each vial with caps replaced loosely to allow aeration. Adding water activated the inoculum (Miles and Wilcoxin 1984). 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 hours fluorescent light. Each isolate was evaluated for ability to cause germinant disease for 14 days. After 3 days, germinants were first checked for disease and checked daily thereafter. Standard post-emergence damping-off was the most common disease encountered. In some cases, a wet-rot type of disease occurred where the root was decayed but the above-ground portion of the germinant not affected. Diseased seedlings were removed, their roots washed thoroughly and placed directly on Komada's medium for re-isolation of inoculated isolates. After 14 days, 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 as described above.

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 the inoculated test isolate, and primary root growth within vials (James 1996). The range of possible points was 3-23, with higher point values reflecting less virulence by the tested isolate. To convert points to a score in which higher numbers represented greater virulence, a reciprocal rating system was used. In this system, the maximum score was 100 and the minimum score was zero.

Table 1. Characteristics of *Fusarium sporotrichioides* isolates evaluated for pathogenicity on Douglas-fir germinants.

Isolate[1]	Host[2]	Source	Nursery[3]
8813C	WL	Seed	NW
9007I	DF	Healthy Seedling	CDA
9008F	DF	Diseased Seedling	CDA
9114A	---	Peat/Vermiculite Media	PC
9114C	---	Peat/Vermiculite Media	PC
9114G	---	Peat/Vermiculite Media	PC
9117A	---	Peat/Vermiculite Media	PEN
9148K	PP	Diseased Seedling	PC
9219-O	DF	Seed	UI
9244I	DF	Diseased Seedling	COL
9307C	---	Styroblock Container	POT
9307D	---	Styroblock Container	POT
9307M	---	Styroblock Container	POT
9309C	PP	Diseased Seedling	RA
9325A	WL	Diseased Seedling	MS
9329F	WL	Diseased Seedling	NW
9330D	WP	Healthy Seedling	POT
9502I	WP	Diseased Seedling	CDA
9525A	---	Soil (Bareroot)	CDA
9703D	---	Styroblock Container	UI
9706F	DF	Healthy Seedling	UI

[1] First two numbers indicate year of isolation.

[2] DF = Douglas-fir; PP = ponderosa pine; WL = western larch; WP = western white pine

[3] CDA = USDA Forest Service Nursery, Coeur d'Alene, ID; COL = Colville Indian Reservation Nursery, Nespelem, WA; MS = Montana Department of State Lands Nursery, Missoula, MT; NW = North Woods Nursery, Elk River, ID; PEN = Peninsulab (currently Eden Bioscience, Inc.), Poulsbo, WA; PC = Plum Creek Nursery, Pablo, MT; POT = Potlatch Nursery, Lewiston, ID; RA = Raintree Nursery, Libby, MT; UI = University of Idaho Research Nursery, Moscow, ID.

Table 2. Virulence of selected *Fusarium sporotrichioides*, *F. proliferatum*, and *F. oxysporum* isolates on Douglas-fir germinants.

Isolate[1]	Ave. Virulence Score[2]	Standard Deviation	Virulence Rating[3]
8813C	28.1 A	10.2	Non-Pathogenic
9007I	25.4 A	14.1	Non-Pathogenic
9008F	23.3 A	13.8	Non-Pathogenic
9114A	38.5 A	19.2	Non-Pathogenic
9114C	31.0 A	20.1	Non-Pathogenic
9114G	32.9 A	11.8	Non-Pathogenic
9117A	29.6 A	5.4	Non-Pathogenic
9148K	34.2 A	17.1	Non-Pathogenic
9219-O	27.3 A	27.3	Non-Pathogenic
9244I	19.8 A	18.8	Non-Pathogenic
9307C	31.9 A	15.7	Non-Pathogenic
9307D	30.0 A	7.4	Non-Pathogenic
9307M	43.1 B	19.6	Low Virulence
9309C	28.8 A	15.9	Non-Pathogenic
9325A	29.1 A	17.4	Non-Pathogenic
9329F	21.3 A	7.4	Non-Pathogenic
9330D	32.1 A	18.2	Non-Pathogenic
9502I	30.2 A	13.4	Non-Pathogenic
9525A	31.5 A	20.7	Non-Pathogenic
9703D	24.4 A	13.6	Non-Pathogenic
9706F	30.8 A	20.2	Non-Pathogenic
9251(FPRO)[4]	50.4 B	23.4	Low Virulence
9431(FPRO)[4]	75.8 C	10.0	Moderate Virulence
Fo47(FOXY)[5]	37.1 A	18.7	Non-Pathogenic
Control	23.1 A	7.7	Non-Pathogenic

[1]First two numbers indicate year of isolation.

[2]Possible ratings range from 0-100; means followed by the same capital letter are not statistically different (P=).05) using Duncan's Multiple Range Comparison Test.

[3]Based on average ratings: 0-40 non-pathogenic; 40-60 low virulence; 60-80 moderate virulence; 80-100 high virulence.

[4]*Fusarium proliferatum:* isolate 9251 obtained from the roots of a non-diseased container-grown Douglas-fir seedling grown at the University of Idaho Research Nursery, Moscow, ID; isolate 9431 obtained from the roots of a diseased bareroot eastern white pine seedling grown at the Griffith State Forest Nursery, Wisconsin Rapids, WI.

[5]*Fusarium oxysporum:* isolate marketed as a biological control agent to control pathogenic *F. oxysporum* causing wilt diseases of agricultural crops; obtained from C. Alabouvette (Dijon, France).

Table 3. Infection, disease, and survival of Douglas-fir germinants inoculated with selected *Fusarium sporotrichioides, F. proliferatum* and *F. oxysorum* isolates.

Isolate[1]	Percent Diseased[2]	Average Survival[3]	Average Infection[4]
8813C	25	14.0	92
9007I	13	13.7	79
9008F	4	13.7	63
9114A	21	12.8	96
9114C	17	13.1	92
9114G	17	13.7	96
9117A	4	14.0	83
9148K	21	13.0	63
9219-O	21	12.5	92
9244I	8	13.4	54
9307C	8	13.5	100
9307D	4	13.5	92
9307M	42	12.5	96
9309C	25	13.7	96
9325A	21	13.4	29
9329F	4	14.0	100
9330D	17	13.4	88
9502I	4	13.5	88
9525A	21	13.1	88
9703D	4	13.5	92
9706F	54	12.7	88
Average	14.6	11.7	73.4
9251(FPRO)[5]	100	7.9	100
9431(FPRO)[5]	45	11.1	100
Fo47(FOXY)[6]	21	12.8	100
Control	16	14.0	92[7]

[1]First two numbers indicate year of isolation.

[2]Percent of the 24 inoculated germinants than became diseased (damping-off or radicle decay).

[3]Average number of days inoculated germinants survived (maximum = 14 days).

[4] With of the 24 inoculated germinants that became infected with inoculated isolates.

[5] *Fusarium proliferatum*: isolate 9251 obtained from the roots of a non-diseased container-grown Douglas-fir seedling grown at the University of Idaho Research Nursery, Moscow, ID; isolate 9431 obtained from the roots of a diseased bareroot eastern white pine seedling grown at the Griffith State Forest Nursery, Wisconsin Rapids, WI.

[6]*Fusarium oxysporum:* isolated marketed as a biological control agent to control pathogenic *F. oxysporum* causing wilt diseases of agricultural crops; obtained from C. Alabouvette (Dijon, France).

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